PB86-171444

Effects of Variable Hardness, pH, Alkalinity, Suspended Clay, and Humics on the Chemical Speciation and Aquatic Toxicity of Copper

(U.S.) Environmental Research Lab.-Duluth, MN

Mar 86

U.S. Department of Commerce
National Technical Information Service
NTIS

PB86-171444

EPA/600/3-86/023
March 1986

THE EFFECTS OF VARIABLE HARDNESS, pH, ALKALINITY, SUSPENDED CLAY, AND HUMICS ON THE CHEMICAL SPECIATION AND AQUATIC TOXICITY OF COPPER

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TECHNICAL REPORT DATA

(Please read Instructions on the reverse before completing)

1. REPORT NO. EPA/600/3-86/023	2.	3. RECIPIENT'S ACCESSION NO.
4. TITLE AND SUBTITLE The Effects of Variable Hardness, pH, Alkalinity, Suspended Clay, and Humics on the Chemical Speciation and Aquatic Toxicity of Copper.		5. REPORT DATE March 1986
		6. PERFORMING ORGANIZATION CODE
7. AUTHOR(S) Henry Nelson*, Duane Benoit, Russell Erickson, Vince Mattson, Jim Lindberg.		8. PERFORMING ORGANIZATION REPORT NO.
9. PERFORMING ORGANIZATION NAME AND ADDRESS * Scinece Applications Int'l.		10. PROGRAM ELEMENT NO.
		11. CONTRACT/GRANT NO.
12. SPONSORING AGENCY NAME AND ADDRESS U.S. Environmental Protection Agency Environmental Research Laboratory-Duluth 6201 Congdon Boulevard Duluth, MN 55804		13. TYPE OF REPORT AND PERIOD COVERED
		14. SPONSORING AGENCY CODE EPA/600/03

15. SUPPLEMENTARY NOTES

16. ABSTRACT

The effects of variable hardness, pH, alkalinity, humics, and suspended clay on the chemical speciation of copper and its toxicity to fathead minnow larvae in Lake Superior water were investigated. Two proposed methods (toxicity factors and chemical speciation) for predicting LC50 values in specific natural waters from laboratory toxicity data and the average site-specific values of general water quality parameters were evaluated. The accuracy of the cupric ion selective electrode in determining CU^{+2} activities in ambient and chemically altered Lake Superior water was also determined.

17. KEY WORDS AND DOCUMENT ANALYSIS

a. DESCRIPTORS	b. IDENTIFIERS/OPEN ENDED TERMS	c. COSATI Field/Group

18. DISTRIBUTION STATEMENT Release to public.	19. SECURITY CLASS *(This Report)* Unclassified	21. NO. OF PAGES 132
	20. SECURITY CLASS *(This page)* Unclassified	22. PRICE

EPA Form 2220-1 (Rev. 4-77) PREVIOUS EDITION IS OBSOLETE

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ABSTRACT

The effects of variable hardness, pH, alkalinity, humics, and suspended clay on the chemical speciation of copper and its toxicity to fathead minnow larvae in Lake Superior water were investigated. Two proposed methods (toxicity factors and chemical speciation) for predicting LC50 values in specific natural waters from laboratory toxicity data and the average site-specific values of general water quality parameters were evaluated. The accuracy of the cupric ion selective electrode in determining Cu^{+2} activities in ambient and chemically altered Lake Superior water was also determined.

Increases in calcium and magnesium hardness at constant ambient Lake Superior alkalinity (approximately 1×10^{-3} eg/L) increased LC50 values in terms of total, dissolved, and free copper (Cu^{+2} activity) as did increases in sodium. Increases in pH from 6.6 to 8.7 at ambient Lake Superior alkalinity increased the total and dissolved copper LC50s. However, the free copper LC50 increased from pH 6.6 to 7.3, remained relatively constant from pH 7.3 to 8.0, and then decreased from pH 8.0 to 8.7. At approximately three times the ambient Lake Superior alkalinity (3×10^{-3} eg/L), the total and dissolved copper LC50s increased monotonically, and the free copper LC50 decreased monotonically with increasing pH from 7.1 to 8.5. Increases in alkalinity from approximately one-third to six times the ambient alkalinity of Lake Superior did not significantly affect the total and dissolved copper LC50s but decreased the free copper LC50s.

The differences between LC50 values for some waters with higher than ambient Lake Superior alkalinity and/or pH appear to be due primarily to changes in the proportions of inorganic copper species with different toxicities/unit concentration. However, the differences between LC50 values for waters with lower than ambient alkalinity and/or pH and for waters at ambient alkalinity and pH but different hardnesses cannot be explained by changes in the proportions of inorganic species. In such cases, it appears likely that changes in general water quality parameters such as pH and hardness, change the toxicity/unit concentrations of one or more toxic copper species.

Additions of humics and/or suspended clay to Lake Superior water increased total and dissolved copper LC50s, but generally decreased free copper LC50s even though they did not have any appreciable affect on pH or alkalinity. The decrease in the free copper LC50 with additions of humics and/or clay may have at least been partially due to increases in the toxicities/unit concentrations of inorganic copper species due to a possible increase in the stress on the organisms. However, it is also possible that some copper humic and/or copper clay complexes are directly toxic to fathead minnow larvae.

The apparent changes in the toxicities/unit concentration of toxic copper species with changes in the values of general water quality parameters such as pH or alkalinity indicates that the feasibility of developing a chemical speciation method for deriving site-specific criteria is low. The development of a toxicity factors method, which involves empirically deriving from laboratory data multi-variable equations relating LC50 values to general water quality parameters such as pH and hardness, appears to be more feasible but is still being evaluated.

The ratio of Cu^{+2} activities determined by the ion selective electrode to Cu^{+2} activities predicted from inputing dissolved copper into the REDEQL chemical equilibrium computer program (assuming no organic or clay complexation) varied from 0.85 to 1.15 for 12 of the 18 test waters to which no humics or clay were added. The four test waters for which the ratios were less than 0.85 had ambient Lake Superior or lower pH and/or alkalinity. Most of the test waters for which the ratios were close to one had greater than ambient Lake Superior pH and/or alkalinity. These observations along with the observed dependence of the slope of the cupric ion electrode response on various parameters indicated that a substantial proportion of copper may be bound by organics in Lake Superior water at ambient or lower pH and/or alkalinity even though the TOC of Lake Superior water averages only 1 ppm. The reason may be due to the relatively high stability constants for the formation of many copper-organic complexes, and to a reduction in the competition between OH^-, CO_3^{-2}, and organic ligands for Cu^{+2} at ambient or lower pH and/or alkalinity.

ACKNOWLEDGEMENTS

We would like to thank the following individuals for their assistance:

1. Charles Stephan, who was responsible for the design of the flow-through electrode chamber, and with whom we held many helpful discussions on the use, calibration, and behavior of the cupric ion electrode.

2. Ron Carlson, who conducted the bioassay work for the part of the Connecticut field study concerning electrode determinations of LC50s, and with whom we hald helpful discussions on the interpretation of the field study data. Mr. Carlson is currently writing a much larger, more detailed report on the Connecticut field study.

3. Robert Andrews, with whom we held several helpful dicsussions on the use, calibration, and behavior of the cupric ion electrode.

5. Ed Leonard and Don Ruppe, who performed some of the atomic absorption analyses of total and dissolved metals.

This research was partially funded under EPA contract No. 68-01-6388, which Science Applications International Corporation (formerly JRB Associates) had with the Office of Water, Criteria and Standards Division, U.S. Environmental Protection Agency, Washington, D.C. This report constitutes the final product of work assignment 16 under this contract.

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1. INTRODUCTION

The aquatic toxicities of metals have been shown to be at least partially dependent upon the values of general water quality parameters such as temperature, dissolved oxygen, hardness, pH, alkalinity, total organic carbon (TOC), and suspended solids (1-20). Since the average values of such parameters can vary substantially over the range of natural waters normally encountered (21), there is interest in formulating protocols by which site specific criteria for metals can be derived (22). EPA is currently evaluating the feasibility of developing protocols for formulating site specific criteria based on non-site laboratory toxicity data bases and on average site specific values of general water quality parameters. Such protocols are potentially more cost effective than those based on performing toxicity tests at each site.

The development of protocols for site specific criteria depends upon the determination of which water quality parameters significantly affect metal toxicity. It also depends upon the development of one or more methods by which the toxicity of metals in a site water can be estimated (e.g., the LC50 values for an appropriate sensitive species) from non-site laboratory toxicity data bases and average site specific values of those general water parameters that significantly affect toxicity.

For the past year, scientists at the EPA lab in Duluth have been conducting toxicity tests to determine the effects of five general water quality parameters (hardness, pH, alkalinity, humics, and suspended solids) on the toxicity of copper to fathead minnow (Pimephales promelas) larvae. The tests were designed to determine which of those general water quality parameters exert a significant effect on copper toxicity. In addition, the tests are being used to evaluate the feasibility of predicting copper toxicity for fathead minnow larvae in site waters from the results of laboratory toxicity tests and the average site specific values of the general water quality parameters found to significantly effect toxicity.

Currently, scientists at the lab are evaluating two proposed methods for estimating copper LC50 values in site water:

a) Toxicity Factors Method - involves empirically deriving one or more equations relating the LC50 or a LC50 related transformation to one or more general water quality parameters (or transformations) such as pH, and hardness. This method is described in Section 1.3.

b) Chemical Speciation Method - involves determining the relative concentration and toxicity/unit concentration of the various copper species present. This method is described in Section 1.4.

Brief summaries of the literature on the chemical speciation of copper in freshwater systems and the effects of chemical speciation on copper toxicity are presented in Sections 1.1 and 1.2, respectively.

1.1 THE CHEMICAL SPECIATION OF COPPER IN FRESHWATER SYSTEMS

The chemical speciation of copper in actual and model freshwater systems has been predicted by numerous authors (6-13,23-36) based on the use of chemical equilibrium computer programs such as REDEQL. In addition, several groups have also based their calculations of the concentrations of various copper species on the actual experimental determinations of Cu^{+2} activities with a cupric ion selective electode (6,8,32-36). We have also based our calculations of the concentrations of copper species in toxicity test waters on cupric electrode determinations of Cu^{+2} activities (Section 1.4). However, for comparative purposes, calculations of copper species concentrations were also performed by inputing dissolved copper determinations into the REDEQL chemical equilibrium computer program.

The relative proportions of the free Cu^{+2} and various copper hydroxy and copper carbonate complex concentrations depend upon the pH and alkalinity of the system and the magnitudes of the stability constants for the formation of the complexes. Although the pH and alkalinity of freshwater systems vary substantially (21), most authors have predicted that the chemical species Cu^{+2}, $CuOH^{+}$, $Cu(OH)_2°$, $CuCO_3°$, and $Cu(CO_3)_2^{-2}$ together represent greater than 98% of the dissolved inorganic copper in the typical freshwater systems they have considered (6-13, 23-26, 32-35). Those conclusions concur with our chemical speciation calculations based on both cupric electrode determinations of Cu^{+2} activities and on REDEQL calculations for the toxicity test waters described later in this report. Although we considered such chemical species as $Cu_2(OH)_2^{+2}$, $CuHCO_3^+$, $CuSO_4$ and various copper chloride, anionic copper hydroxy, copper ammonia and copper phosphate species, the calculated contributions of all such species to the total inorganic copper in the toxicity test waters were negligible (individually <1%, together <2%).

The total dissolved copper in many freshwater systems may also consist of substantial amounts of copper-organic complexes including complexes with amino acids, carboxylic acids, humic acids, and various effective copper chelators (24, 25, 27, 31, 33, 35-54). Although chelators such a NTA and EDTA do not appear to occur naturally (37), they can be introduced to freshwater systems by industrial and municipal sewage discharges (37, 38, 55). Furthermore, there is evidence that aquatic organisms such as algae and some types of invertebrates and fish secrete substantial quantities of chelating agents in response to copper stress (37, 56).

The stability constants for the formation of many copper-organic complexes and the extent of copper binding by many types of humics (37) appear to be of sufficient magnitude to possibly result in substantial copper-organic complexation, even in waters with relatively low organic content (24, 35, 39). However, there is still substantial debate over the concentrations and types of organics required to complex substantial amounts of copper (37). The TOC of Lake Superior water (which served as the base and diluent water for all of our in lab toxicity tests) is on the order of only 1 ppm (57). However, the magnitude of the greater than Nernstian slopes of the cupric ion electrode response along with theoretical REDEQL calculations using glycine as a model of the average organic nitrogen present in Lake Superior water indicate that substantial copper-organic complexation may occur in Lake Superior water and Lake Superior water with lowered pH and/or alkalinity, as will be further discussed in Section 3.10.

The total copper in some freshwater systems substantially exceeds the dissolved copper due to copper precipitation and adsorption to or binding by suspended solids and colloids (33). We have attempted to determine which solids control the copper solubility in our toxicity test waters by considering the pH and alkalinity of the test waters, and the solubility products as listed in Martel and Smith 1976 (58) for the following solids: $CuO_{(s)}$ (tenorite), $Cu_2(OH)_2CO_{3(s)}$ (malachite), and $Cu_3(OH)_2(CO_3)_{2(s)}$. Our calculations indicated that $CuO_{(s)}$ controlled the solubility of copper for all of our toxicity test waters and that the copper solubility decreased with increasing pH over the pH range of our toxicity tests. Our calculations also indicate that the highest concentration of total copper used in each type of toxicity test water was below the calculated total solubility of copper which was defined as the estimated sum of the Cu^{+2}, $CuOH^{+}$, $Cu(OH)_2^{\circ}$, $CuCO_3^{\circ}$ and $Cu(CO_3)_2^{-2}$ concentrations at the saturation point.

Differences between total and dissolved copper in freshwater systems may also be due to adsorption onto various types of suspended solids and colloids including hydrous iron and manganese oxides (59), clays (60, 61), aluminum and silica oxides (24, 62) and humic material (35-54). The adsorption of copper onto suspended clays and oxides generally increases with increasing pH (60-62). Increases in the organic content of sediments and suspended solids also appear to increase copper adsorption (63). Although increases in the overall organic content of the water are reported to enhance the adsorption of copper onto suspended clays and oxides by some groups (60-62), increases in the dissolved organic content of water is reported to greatly reduce such adsorption (35, 64). The apparent discrepancy has been attributed to differences between the effects of suspended or colloidal organic matter and dissolved organics (65). Lake Superior water has a very low suspended solid content so that adsorption of copper onto suspended material was relatively low in all of our toxicity test waters which did not have humic or clay additions. That was demonstrated by the small differences (eg., <15%) between dissolved and total copper measurements. The addition of Lake Superior shore clay and of Aldrich soil derived humic acid did lead to substantial adsorption of the copper onto suspended solids and colloids. The increase in the difference between total and dissolved copper with humic additions indicated that some of the humic material remained in suspension either as particulate matter or as colloidal material.

1.2 THE EFFECTS OF GENERAL WATER QUALITY PARAMETERS AND CHEMICAL SPECIATION ON COPPER TOXICITY TO FISH

Our data (Chapter 3) along with most of the data in the literature indicate that increases in hardness, alkalinity, pH, humic content, TOC in general, and suspended solids generally decrease copper toxicity to fish (increase LC50 values and chronic level values in terms of total and dissolved copper) (1-20). The observed changes in total and dissolved copper toxicities with changes in the values of general water quality parameters can probably be primarily attributed to:

a) Changes in the relative concentrations of different copper species which may exert different toxicities/unit concentration on the test organisms

and/or

b) Changes in the toxicity/unit concentration exerted by toxic copper species on the test organisms which could be reflective of physiological changes within the organisms and/or changes in copper transport rates into and out of the organisms.

Although not completely conclusive, our toxicity data tends to support the postulate that hardness affects total and dissolved copper toxicity primarily through mechanism b, that alkalinity effects are primarily through mechanism a, and that pH, humic and suspended clay effects are through both mechanism a and b.

A number of groups have previously attempted to determine the relative contributions of different copper species to copper toxicity to fish by comparing the results of copper toxicity tests in terms of total or dissolved copper with the corresponding calculated concentrations of the various possible copper species (6-16). The results of such analyses have not been completely conclusive and are not always in agreement. Difficulties with these types of analyses in the past have included a statistically small number of data points compared to the number of chemical species considered and substantial correlations (e.g. lack of independence) between the concentrations of the different chemical species as discussed by Magnuson et al., 1979 (12). At least part, but generally not all of the correlation between the concentrations of some of the different chemical species can be eliminated by varying the values of general water quality parameters such as hardness, alkalinity and pH independently of one another as we have done for the sets of experiments described in this report. However, in addition to affecting the relative concentrations of different chemical species, changes in the values of general water quality parameters such as pH may also change the toxicity/unit concentration exerted by one or more of the toxic copper species on the organism (15). Our data tends to support that postulate as will be discussed in Chapter 3 and 4. Therefore, it may not be possible to quantitatively determine the relative toxicities/unit concentration of different chemical species whose relative concentrations depend upon the values of general water quality parameters that also affect the toxicities/unit concentration.

Despite difficulties with quantitative analyses, the groups who have tried to determine the relative toxic contributions of different copper species have, at least qualitatively, attempted to identify those copper species which do, and do not, contribute significantly to copper toxicity (6-16). Either computer estimated or electrode determined Cu^{+2} activities have been correlated with copper toxicity to fish by all of the groups (6-16). In addition, most have suggested that $CuOH^{+}$ probably also contributes significantly to toxicity (7, 9-14). There is no general agreement on the contribution of $Cu(OH)_2^{\circ}$ to toxicity perhaps at least partially due to the greater than 10^4 range in the reported magnitude of the stability constant for its formation (35,66). The species $CuCO_3^{\circ}$ and $Cu(CO_3)_2^{\circ}$ have generally been described as contributing very little, if any, to toxicity based on a lack of correlation between the concentrations of those species and copper toxicity (7-16). However, one group has suggested that $CuCO_3^{\circ}$ can contribute significantly to copper toxicity when it constitutes a high proportion of the copper present (6). Copper bicarbonate $CuHCO_3^{+}$ and the anionic copper hydroxy species such as $Cu(OH)_3^{-}$ and $Cu(OH)_4^{-2}$ are not believed to contribute significantly to copper toxicity based on either a lack of correlation between predicted concentration and copper toxicity, or to the low level of the predicted concentrations (6-16). Although several groups have suggested that $Cu_2(OH)_2^{+2}$ may be toxic (9, 10, 11), the predicted concentration of that species in most freshwater systems appears to be too low to contribute significantly to toxicity (11).

Humic acid (17, 18, 20), NTA (6), EDTA (16), glycine (16, 20), citrate (16), and municipal sewage effluent (67) have all been reported to reduce the toxicity of copper to fish in terms of total and/or dissolved copper. However, it is still possible, as pointed out by Borgmann 1983 (15), that copper-organic complexes do exert some toxicity to fish, although lower than that exerted by Cu^{+2} and perhaps other toxic inorganic copper species. Although constant LC50 values in terms of free copper (Cu^{+2}) in the presence and absence of such organics would indicate that the copper-organic complexes were non-toxic, a reduction in the Cu^{+2} $LC50_s$ would indicate that the copper-organic complexes might be contributing to toxicity (15).

There is some evidence which indicates that Cu^{+2} $LC50_s$ remain relatively constant for algae and bacteria in the presence and absence of various kinds of organics (15) although there is little, if any, comparative data of that nature available on fish. However, a recent study on the effects of various amino acids on copper toxicity to the aquatic invertebrate Daphnia indicated that the addition of any of the amino acids tested substantially reduced the Cu^{+2} LC50 (68). Although that indicates that the copper-amino acid complexes may contribute to toxicity, the increase in total copper $LC50_s$ with the amino acid additions indicates that the toxicity/unit concentration exerted by the copper-amino acid complexes is lower than the concentration weighted average toxicity/unit concentration exerted by the inorganic copper species. Qualitatively similar results were obtained with guppies, but the apparent toxicity/unit concentration of the copper-amino acid complex was far less than for the Daphnia (68). Our toxicity data indicate that humic additions increase total and dissolved copper LC50s, but decrease Cu^{+2} $LC50_s$. Therefore, the copper-humic complexes may exert some toxicity, but with a lower toxicity/unit concentration than the concentration weighted average toxicity/unit concentration of the inorganic copper species.

The toxicity of copper precipitates and copper adsorbed onto suspended solids has not been well characterized, but the small amount of data available indicates that those forms of copper are probably relatively non-toxic compared to comparable quantities of some dissolved forms of copper (9, 16, 69). However, our data on the effects of suspended clay on copper toxicity indicate that Cu^{+2} $LC50_s$ are reduced by suspended clay additions which indicates that copper-clay complexes might exert some toxicity to fathead minnows.

1.3 TOXICITY FACTORS METHOD

The toxicity factors method involves estimating LC50 values in site waters by first empirically developing an equation relating the LC50 value in terms of an appropriate measurable form of copper to general water quality parameter variables through multivariable regression on laboratory toxicity data. Average site specific values of the general water quality parameters are then substituted into the equation. An example of such an equation can be found in Howarth and Spraque 1979 (10).

The selection of an appropriate measurable form of copper should be based upon the ease at which the measurement can be made in natural waters and upon how closely the measured form approximates the bioavailable fraction of copper. The measurable forms of copper whose LC50 values are dependent upon the least number of general water quality parameters will be the ones which most closely approximate the bioavailable fraction of copper. Measurable forms of copper which have or will be considered in the future include total copper, 0.45 um filtered (dissolved) copper, copper fractions with molecular weights $\leq$ 1,000 as determined by dialyses or ultrafiltration and uncomplexed "free" copper as determined by Cu^{+2} activity measurements with an ion selective electrode.

If an equation relating LC50 values in terms of a chosen measurable form of copper to general water quality parameters is developed from laboratory toxicity data, the accuracy of the equation for estimating LC50 values in site waters can be tested by comparing equation estimated LC50 values to LC50 values determined from toxicity tests conducted at several different sites that are representative of the wide range of natural waters normally encountered.

1.4 CHEMICAL SPECIATION METHOD

The chemical speciation method of estimating LC50 values in site waters involves determining the relative contributions of different soluble species of copper to the overall toxicity of the copper in the site waters. Assuming additive toxicity, the contribution of a particular soluble species of copper to the overall toxicity of copper in a site water will depend upon the product of the concentration of the chemical species times the toxicity that would be exerted by the chemical species alone per unit concentration. Before describing the chemical speciation method in more detail, it will be necessary to briefly discuss chemical speciation calculations and the concepts of toxicity units and toxicity per unit concentration.

The major soluble inorganic species of copper in most natural waters are Cu^{+2}, $CuOH^{+}$, and $CuCO_3^{\circ}$ usually followed to a lesser extent by $Cu(OH)_2^{\circ}$ and $Cu(CO_3)_2^{-2}$ (Section 1.1). If an LC50 value in terms of the Cu^{+2} activity is determined in a given type of test water from ion selective electrode measurement of Cu^{+2} activities, the concentrations of the major inorganic species at the LC50 point can be calculated from the following equations:

$$[Cu^{+2}]_{LC50} = (Cu^{+2})_{LC50}/\gamma_{Cu^{+2}} \qquad (1\text{-}1)$$

$$[Cu(OH)^{+}]_{LC50} = K_{CuOH}\, K_w (Cu^{+2})_{LC50}\, 10^{pH}/\gamma_{CuOH^{+}} \qquad (1\text{-}2)$$

$$[Cu(OH)_2^{\circ}]_{LC50} = B_{Cu(OH)}\, K_w^{2} (Cu^{+2})_{LC50} 10^{2pH}/\gamma_{Cu(OH)_2^{\circ}} \qquad (1\text{-}3)$$

$$[CuCO_3^{\circ}]_{LC50} = K_{CuCO_3}\ (Cu^{+2})_{LC50}\ (CO_3^{-2})/\gamma_{CuCO_3} \qquad (1\text{-}4)$$

$$[Cu(CO_3)_2^{-2}]_{LC50} = B_{Cu(CO_3)_2^{-2}}\ (Cu^{+2})_{LC50}\ (CO_3^{-2})^2/\gamma_{Cu(CO_3)_2^{-2}} \qquad (1\text{-}5)$$

where

$(Cu^{+2})_{LC50}$ = the LC50 point in terms of the Cu^{+2} activity at the LC50 point

$$(CO_3^{-2}) = \frac{\text{Alkalinity} - K_w 10^{pH}/\gamma_{OH^-} + 10^{-pH}/\gamma_{H^+}}{2/\gamma_{CO_3^{-2}} + 10^{pH}/K_{a2}\,\gamma_{HCO_3^-}} \qquad (1\text{-}6)$$

K_{CuOH^+}, $B_{Cu(OH)_2}$, $K_{CuCO_3^{\circ}}$, $B_{Cu(CO_3)_2^{-2}}$ = stability constants

K_w = ion product for water
K_{a2} = acid dissociation constant for HCO_3^-
= activity coefficients

Likewise, the concentration of any other soluble monomeric copper species at the LC50 point, organic or inorganic, which can be represented as being formed from Cu^{+2} can be calculated from the general equation

$$[CuL_j^{2-wj}]_{LC50} = K_{CuL_j} (Cu^{+2})^i_{LC50} (L^{-w})^{j/} CuL_j \quad (1-7)$$

where

(L^{-w}) = activity of ligand with charge -w

The accuracy of the above calculations using equations (1-1) to (1-7) will depend to a large extent on how close the copper species are to equilibrium with the Cu^{+2} which is actually at steady state in the flow through systems, not equilibrium. This problem will be discussed in greater detail in Section 3.9.

The activity coefficients for neutral species in dilute solutions are approximately equal to 1 so $\gamma_{CuCO_3^{\circ}}$ and $\gamma_{Cu(OH)_2^{\circ}}$ are assumed to be equal to 1 in the above equations. The activity coefficients for charged species can be estimated from the Davies equation

$$Z = 10^{[-.51Z^2/(1+ I) + .3I]} \quad (1-8)$$

where

Z = species charge
I = ionic strength

The ionic strength can be estimated from the hardness and alkalinity of the solutions and concentrations of any added electrolytes as will be discussed in Section 2.11.

The toxicity of a copper solution can be expressed in terms of toxicity units (70) where 1 toxicity unit is defined as the amount of toxicity requir to kill 50% of the test organisms during a 96 hr exposure. Therefore, by definition, the toxicity of a copper solution at the LC50 point (e.g., a copper solution in which 50% of test organisms will be killed during a 96 hr exposure) is equal to one toxicity unit. The fractional contribution of any given toxic copper species to the 1 toxic unit at the LC50 point will, assuming additive toxicity, be equal to the product of the concentration of the species at the LC50 point times the number or fraction of toxicity units that the toxic species would exert if it was alone in solution at unit concentration (e.g., 1 molar). Therefore, assuming additive toxicity and that all toxicity is due to dissolved copper species, the following equation should be valid at the LC50 point for any type of test water (70).

$$1 = T_1[Cu^{+2}]_{LC50} + T_2[CuOH^{+}]_{LC50} + T_3[Cu(OH)_2^{\circ}]_{LC50} + T_4\ [CuCO_3^{\circ}]_{LC50} + T_5[Cu(CO_3)_2^{-2}]_{LC50} + \ldots\ldots T_n[Cu_iL_j^{2i-wj}]_{LC50} \qquad (1\text{-}9)$$

where

T_1 = number or fraction of toxicity units which would be exerted by Cu^{+2} if it was alone in solution at a unit concentration (e.g., toxicity/unit concentration of Cu^{+2} or alternatively the inverse of the LC50 in terms of Cu^{+2} if all of the copper was in the form of Cu^{+2})

T_2 = toxicity/unit concentration of $CuOH^{+}$

T_3 = toxicity/unit concentration of $Cu(OH)_2^{\circ}$

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T_n = toxicity/unit concentration of Cu_iL_j

In theory the right side of equation (1-9) should include all souble chemical species which contribute to the overall toxicity of the solution. In practice, the left side of equation (1-9) can be approximated by including on the right side only those species which might contribute significantly (e.g. >1%) to toxicity.

The chemical speciation method of estimating Cu^{+2} LC_{50} values in site waters depends upon first using multiple linear regression analyses to mine the values of the toxicity/unit concentration (e.g. T_1, T_2, T_3, ... T_n) exerted by all of the chemical species which contribute significantly to toxicity. Substituting equations (1-1) to (1-7) into equation (1-9) and then dividing both sides of the equation by the Cu^{+2} activity gives an equation which is more suitable for multiple linear regression analyses than equation (1-9).

$$1/Cu^{+2}_{LC50} = T_1 x_1 + T_2 x_2 + T_3 x T_3 + T_4 x_4 + T_5 x_5 + \ldots T_n x_n \qquad (1\text{-}10)$$

where

$$x_1 = 1/\gamma_{Cu^{+2}}$$

$$x_2 = K_{CuOH} \; K_w 10^{pH}/\gamma_{CuOH^+}$$

$$x_3 = B_{Cu(OH)_2} \; K_w^2 \; 10^{2pH}$$

$$x_4 = K_{CuCO_3} \left[\frac{Alk - K_w 10^{pH}/\gamma_{OH^-} + 10^{pH}/\gamma_{H^+}}{2/\gamma_{CO_3^{-2}} + 10^{-pH}/K_{a2}\gamma_{HCO_3^-}}\right]$$

$$x_5 = B_{Cu(CO_3)_2^{-2}} \left[\frac{Alk - K_w \; 10^{pH}/\gamma_{OH^-} + 10^{-pH}/\gamma_{H^+}}{2/\gamma_{CC_3^{-2}} + 10^{-pH}/K_{a2}\gamma_{HCO_3^-}}\right]^2 \Bigg/ \; \gamma_{Cu(CO_3)_2^{-2}}$$

$$x_n = K_{CuL_j} \; (L)^j/\gamma_{CuL_j}$$

The toxicity/unit concentration (T_1, T_2, T_3 ... T_n) exerted by each of n chemical species can be theoretically determined by

a) Determining the inverse of LC50 values in terms of the Cu^{+2} activity for $N>n$ types of test water for which the relative concentrations of the n species change, but the toxicities/unit concentration of the n species remain constant

b) Calculating the values of x_1, x_2, x_3, ... x_n of equation (1-10) from the values of the pH and alkalinity at the LC50 point for each of the N test waters

c) Performing multiple linear regression of the inverse of the Cu^{+2} LC50 on the variables x_1, x_2, x_3 ... x_n using the data from the $N>n$ test waters to determine the values of the toxicities/unit concentration (T_1, T_2, T_3, ... T_n)

If the toxicities/unit concentration (T_1, T_2, T_3, ... T_n) can be determined, the Cu^{+2} LC50 in a given site water can be estimated by:

a) Determining the average site specific values of pH, alkalinity, and hardness

b) Estimating the values of activity coefficients in the site water from the substitution of the estimated site water ionic strength (based primarily on site water alkalinity and hardness) into the Davies equation (1-8)

c) Substituting the toxicities/unit concentration, the estimated activity coefficient, and the average site specific values of pH and alkalinity into equation (1-10) and solving for the Cu^{+2} activity at the LC50 point.

If the Cu^{+2} LC50 can be estimated for a site water, the total copper and dissolved copper LC50s can be estimated by titrating a sample of the site water with copper until the activity of Cu^{+2} as determined with a cupric ion electrode equals the estimated Cu^{+2} LC50. The titrated sample can then be analyzed by atomic absorbtion spectroscopy to determine the total and dissolved copper in the sample which will correspond to the estimated total and dissolved copper LC50s for the site water.

1.5 USEFUL EQUATIONS FOR INTERPRETATION OF TOXICITY TEST DATA

Before discussing the results of the toxicity tests, it might be helpful to define a few terms and to transform a more generalized form of equation I-9 to a couple of other forms. Equation I-9 equates the one toxicity unit at the LC50 point to the sum of the product of the toxicity/unit concentration T_i of each toxic species i times its concentration at the LC50 point $[C_i]_{LC50}$ and is valid for additive toxicity. A more generalized form of equation (1-9) is

$$1 = \sum_i T_i [C_i]_{LC50} \qquad (1\text{-}11)$$

The product of the toxicity/unit concentration of each species i times its concentration at the LC50 point is, assuming additive toxicity, equal to the overall fractional contribution f_i of the particular species to the one toxicity unit at the LC50 point

$$f_i = T_i[C_i]_{LC50} \qquad (1\text{-}12)$$

Substituting equation (1-12) into (1-11) gives

$$1 = \sum_i f_i \qquad (1\text{-}13)$$

Assuming additive toxicity, equation (1-13) is valid for any test water at the LC50 point. Subtracting equation (1-13) for a test water A from equation (1-13) for a test water B gives the following equation which may be useful for comparing the changes in the fractional contribution of various species to toxicity in going from any test water A to any test water B.

$$0 = \sum_i \Delta f_{i(B-A)} \qquad (1\text{-}14)$$

where by considering equation (1-12), it can be seen that

$$\Delta f_{i(B-A)} = T_{iB}[C_i]_{LC50(B)} - T_{iA}[C_i]_{LC50(A)} \qquad (1\text{-}15)$$

If the toxicity/unit concentration of each species i remains constant in going from test water A to B then

$$\Delta f_{i(B-A)} = T_i([C_i]_{LC50(B)} - [C_i]_{LC50(A)}) \qquad (1\text{-}16)$$

and substituting equation (1-16) into (1-14) gives

$$0 = \sum_i T_i \, \Delta[C_i]_{LC50(B-A)} \qquad (1\text{-}17)$$

An equation relating the change in the LC50 in going from test water A to B, to changes in the sum over all toxic species of the product of the toxicity/unit concentration times the proportion of dissolved copper represented by the species can be derived as follows. The concentration of any copper species i at the LC50 point in any test solution is equal to the product of the proportion of dissolved copper at the LC50 point that is represented by the species times the dissolved copper LC50:

$$[C_i]_{LC50} = P_i(LC50)_{\text{Dissolved Cu}} \qquad (1\text{-}18)$$

Substituting equation (1-18) into equation (1-11) gives:

$$1 = \sum_i T_i P_i (LC50) = (LC50) \sum_i T_i P_i \qquad (1\text{-}19)$$

Rearrangement of equation (1-19) give:

$$LC50 = \frac{1}{\sum_i T_i P_i} \qquad (1\text{-}20)$$

Using equation (1-20) it can be seen that the difference in the dissolved copper LC50 in going from test water A to B is given by:

$$(\Delta LC50)_{(B-A)} = \frac{(\sum_i T_{iA} P_{iA} - \sum_i T_{iB} P_{iB})}{(\sum_i T_{iA} P_{iA})\ (\sum_i T_{iB} P_{iB})}$$

Equation (1-21) relates changes in the dissolved copper LC50s between any two test waters to changes in the products of the toxicity/unit concentration times the proportion of dissolved copper for various copper species. However, it gives no information concerning the separate contribution of proportional changes and any toxicity/unit concentration changes to the changes in the dissolved copper LC50. To do so, it is necessary to take the total differential of equation (1-20) to give

$$dLC50 = \sum_{j=1}^{j=n} \frac{-T_j}{\left(\sum_{i=1}^{i=n} T_i P_i\right)^2} dP_j + \sum_{j=1}^{j=n} \frac{-P_j}{\left(\sum_{i=1}^{i=n} T_i P_i\right)^2} dT_j \qquad (1\text{-}22)$$

Equation (1-22) shows that differential changes in the LC50 value are dependent upon differential changes in the proportion and/or toxicity/unit concentration of the n chemical species contributing to the toxicity of the solution. The first grouped term of equation (1-22) represents the overall contribution of differential proportional changes in toxic chemical species to the differential change in the LC50 value. The second grouped term of equation (1-22) represents the overall contribution of differential changes in the toxicities/unit concentration of toxic chemical species to the differential change in the LC50 value.

It can be seen from the negative signs within the first and second grouped terms of equation (1-22), that a differential decrease in the proportion of a toxic species j will make a positive contribution to the differential change in the LC50 value, as will a differential decrease in the toxicity/unit concentration of the species. Likewise, a differential increase in the proportion of a toxic species j will make a negative contribution to the differential change in the LC50 value, as will a differential increase in the toxicity/unit concentration of the species. The sign and magnitude of the differential change in the LC50 value will be equal to the sum of all positive and negative differential contributions of all n toxic species j.

Although the above discussion concerned differential changes, the qualitative relationship between the signs of proportional and toxicity/unit concentration changes and the sign of a LC50 change should be valid for interval changes as well.

2. METHODS AND MATERIALS

2.1 GENERAL APPARATUS AND MATERIALS FOR Cu^{+2} ACTIVITY AND pH DETERMINATIONS

All Cu^{+2} activity determinations were made with an Orion cupric ion electrode (model 94-29) referenced against an Orion Ag/AgCl double junction reference electrode (model 90-02) (36, 71). The inner chamber filling solution used for the reference electrode was an Orion supplied AgCl solution (90-00-02). The outer chamber filling solution used was an Orion supplied 10% KNO_3 solution (90-00-03). The pH measurements were obtained with either an Orion pH glass electrode (910100) or a Fisher glass pH electrode (13-639-3) referenced against the same Orion double junction reference electrode used to reference the cupric ion electrode. Both pH and Cu^{+2} mV readings were obtained from an Orion 801 meter attached to either a model 605 or 855 electrode switch. The use of an electrode switch allowed the cupric ion and pH electrodes to be referenced against the same reference electrode simultaneously which has diagnostic advantages, and allowed alternate cupric ion electrode mV and pH readings to be made by simple switch adjusment.

2.2 CUPRIC ION AND pH ELECTRODE CALIBRATIONS

Two point pH calibrations were performed daily in pH 7 and pH 10 buffers supplied by Preiser for pH measurements in natural waters, and in pH 7 and pH 4 buffer supplied by MCB for pH measurements in acetate buffer.

The potential of an Orion cupric ion electrode/double junction reference electrode can be theoretically related to the Cu^{+2} ion activity in solution by the Nernst equation (72)

$$E = E' + \frac{2.3\ RT}{2F} \log (Cu^{+2}) \tag{2-1}$$

where

E = potential reading in mV of the electrode pair

E' = constant dependent upon the Ag^+/Ag° standard reduction potential, copper sulfide and silver sulfide solubility products, the reference electrode potential and any associated junction potentials

(Cu^{+2}) = activity of Cu^{+2}

2.3 RT/2F = "Nernstian slope" = 29.3 mV/log unit at 22°C

Although the slope is sometimes theoretically assumed to be equal to the Nernstian slope, the value of E' cannot be theoretically estimated. In practice, both the slope and E' (intercept) are determined experimentally through calibration of the electrode. The calibration of the electrode consists of plotting mV readings versus Cu^{+2} activity in solutions where the Cu^{+2} activity can be independently determined, and then performing linear regression on the data to determine the experimental slope and intercept (E'). If the resultant plot appears linear and the experimental slope is approximately equal to the theoretical slope, the electrode is described as having a "Nernstian" slope or as exhibiting "Nernstian" behavior.

The cupric ion electrode was calibrated in 0.01 M acetate buffer (36, 71, 79) consisting of equal molar amounts of Fisher reagent grade anhydrous sodium acetate and high purity ("Ultrex") acetic acid purchased from Baker. The cupric ion electrode was calibrated in acetate buffer ($pH < 5.0$) to maintain a relatively constant pH with copper addition and to make the contribution of $Cu(OH)_2^\circ$ to the total copper negligible because the reported stability constant values in the literature for that complex vary substantially. However, there is very little disagreement between stability constant values reported for copper acetate complexes.

The calibrations of the cupric ion electrode were carried out in an initial volume of 200 ml acetate buffer in a 250 ml teflon beaker wrapped with electrical tape to shield the light sensitive cupric ion electrode. The solutions were stirred with a teflon stirring bar and the beaker was covered with a commercial opaque teflon electrode holder. The calibrations were performed by making 7-10 additions of a 10^{-3} M standard and 3 additions of a 10^{-1} M standard which gave a Cu^{+2} activity calibration range from approximately 3×10^{-8} M to 10^{-4} M. After each addition of the $CuNO_3$ standard, a stable mV reading was made followed immediately by the withdrawal of 2 ml of the buffer for total copper analysis by atomic absorption spectroscopy.

The Cu^{+2} activity in each 2 ml buffer sample was calculated from the pH and total copper in the buffer using the following two equations:

$$(Cu^{+2}) = \frac{Cu_T}{(1/\gamma_{Cu^{+2}}) + (K_{CuAc}\ (Ac-)/\gamma_{CuAc}) + (B_{CuAc_2}\ (Ac^-)^2)}$$

where

$$(Ac^-) = \frac{Ac_T}{(K_{HAc\ (formation)}\ 10^{-pH}) + (1/\gamma_{Ac^-})}$$

and

(Ac^-) = acetate ion activity

Ac_T = total acetate

K_{CuAc^+}, $B_{Cu(Ac)2}$ = stability constants for copper acetate complexes

K_{HAc} = stability constant for formation of acetic acid

Calibration curves were constructed from linear regressions on mV readings versus Cu^{+2} activities. Calibration curves were almost always linear with approximately Nernstian slopes down to 10^{-7} M Cu^{+2} activity. There was generally some scatter of points below 10^{-7} M, but there were no discernible non-linear trends down to the blank. The blank generally had Cu^{+2} activities $> 1.5 \times 10^{-8}$ M corresponding in .01 M acetate buffer to the total copper detection limit with the atomic absorption spectrometer. The scatter of points below 10^{-7} M Cu^{+2} activity was probably due to a combination of sample contamination and increasing inaccuracy of atomic absorption spectroscopy determinations below 10 ppb. The often reported non-linear behavior of the electrode at Cu^{+2} activities below 10^{-6} M was observed only when mV vs Cu^{+2} activity curves were based on Cu^{+2} activity determinations from nominal total copper instead of total copper determined from atomic absorption spectroscopy. The pseudo non-linear behavior of the electrode when Cu^{+2} activity determinations are based on nominal total copper instead of actual total copper, and the presence of $>1.5 \times 10^{-8}$ M Cu^{+2} activities in blank acetate buffer may be due to oxidative dissolution of copper off the membrane as postulated by several authors (73, 74, 75).

Calibration curves were very reproducible over many weeks. Figure 2-1 shows the typical reproducibility of calibration curves for two calibrations performed over one month apart. The figure also demonstrates the difference between basing calibration curves on Cu^{+2} activities determined from nominal total copper instead of actual total copper (e.g., determined by atomic absorption spectroscopy). The curves based on actual total copper are linear with approximately Nernstian slopes down to the blank, whereas curves based on nominal total copper are non-linear with positive curvature below a Cu^{+2} activity of approximately $5x10^{-7}$M.

The slope and intercept of cupric ion electrode calibration curves were generally obtained from linear regression performed only on data with Cu^{+2} activities above 10^{-7} M due to the usual scatter of data with Cu^{+2} activities below 10^{-7} M. However, many of the Cu^{+2} activities in copper toxicity test solutions were below 10^{-7} M down to as low as 10^{-9} M. Therefore, it was necessary to calculate those activities from linear extrapolations of the calibration curves. Several authors have shown that the Orion cupric ion electrode exhibits linear Nernstian behavior to well below 10^{-10} M Cu^{+2} activity by using copper buffer solutions (76, 77, 78). The errors associated with extrapolation due to uncertainties in the slope should have been reduced by the performance of the linear regression on numerous data points (e.g., 8 or 9) with Cu^{+2} activities above 10^{-7} M.

In retrospect, the accuracy of Cu^{+2} determinations could have possibly been improved by extending calibrations into the range of 10^{-7} to 10^{-9} M by using a copper buffer instead of acetate pH buffer. However, electrode response times in such regions are generally slow and errors introduced by the use of a copper buffer (e.g., buffer complexing interferences with spike recoveries with atomic absorption analyses) may offset any advantage gained by not using extrapolation.

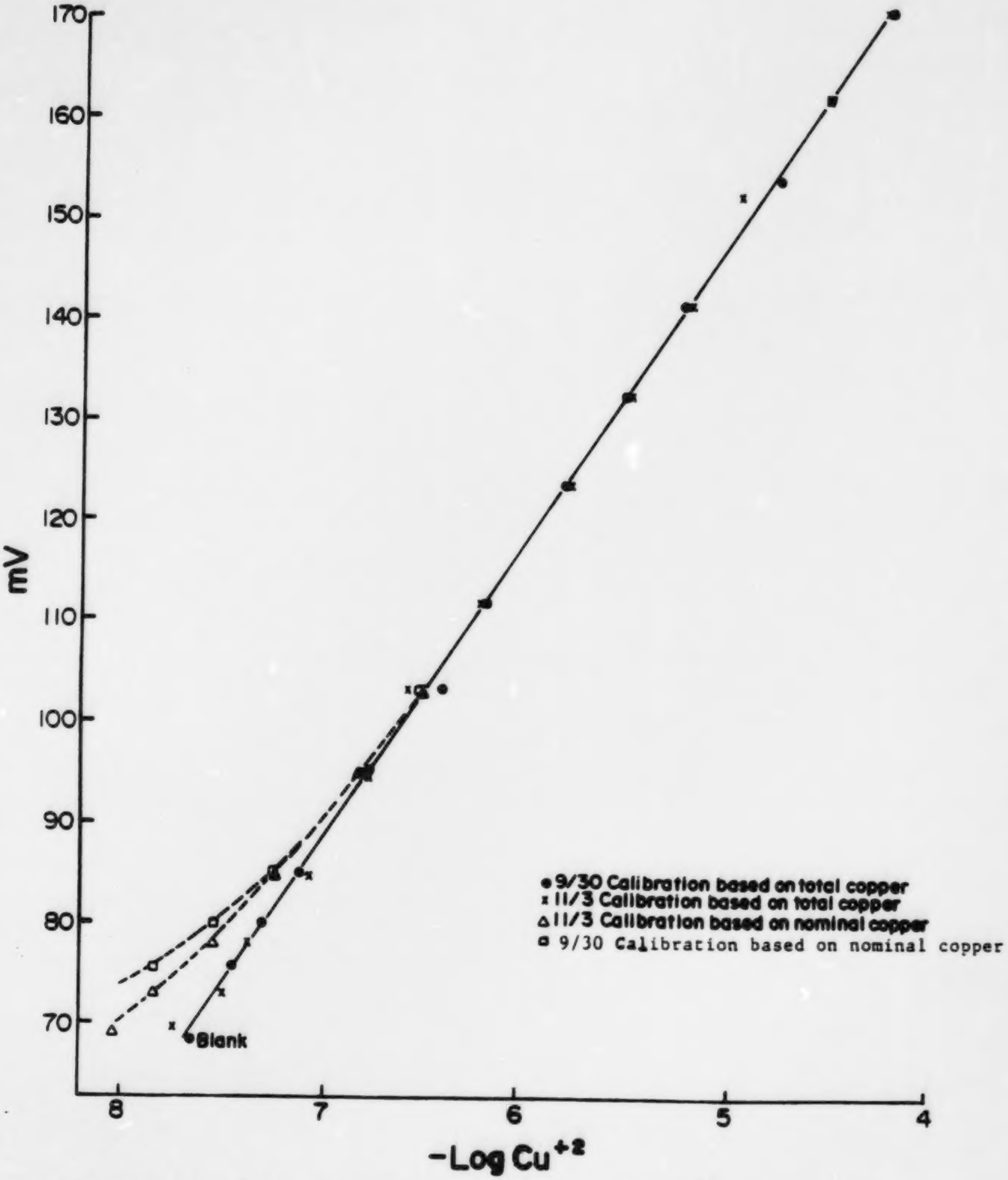

Figure 2-1. A typical calibration curve for the cupric (Cu^{+2}) ion electrode

2.3 Cu^{+2} ACTIVITY AND PH MEASUREMENTS IN FLOW-THROUGH SYSTEMS

All of the laboratory toxicity tests were performed in flow through tanks which will be described in Section 2.5. The Cu^{+2} activity measurements in the flow-through tanks were performed with the use of an electrode chamber based on a design by C. Stephan of the Duluth lab. The electrode chamber was designed to hold the cupric ion, reference, and pH electrodes, shield the cupric ion electrode from light, and to direct all flow in the tank through the electrode chamber. The passage through the electrode chamber and the placement of the cupric ion electrode were designed so that the membrane surface of the electrode was continually impacted by the flow through the chamber.

Since cupric ion electrode measurements require a certain minimal flow, use of the flow through electrode chamber eliminated the necessity of stirring or bubbling which can, in some cases, affect the pH of the test solution. Furthermore, the use of a flow-through electrode chamber should have minimized any errors associated with possible oxidative dissolution of Cu^{+2} off the membrane surface (75). Comparisons of mV readings obtained with the electrode chamber to mV readings obtained with a stirring system in Lake Superior water at various Cu^{+2} activities were within 1 mV corresponding to a difference between measured activities of <8%.

The cupric ion electrode was polished with Orion supplied polishing strips every day before calibration or flow-through tank measurements were taken to remove any surface irregularities or oxidized material (72, 73, 80) from the electrode membrane. The electrode was then thoroughly rinsed with deionized water or with .001 M EDTA/.01 M ascorbic acid (46, 71) and then deionized water. However, rinsing with .001 M EDTA/.01 M ascorbic acid, which is a mild copper reducing and complexing solution, did not appear to have any beneficial affect when following polishing. That concurs with previous observations by others (77) although it appeared to improve the performance of electrodes if they were not polished. The internal and external chambers of the double junction reference electrode were also refilled daily before measurements were taken.

As previously discussed, two point pH calibrations were performed each day prior to measurements. However, due to the reproducibility of cupric ion electrode calibrations over several weeks (see previous section and Figure 2-1), full calibrations of the cupric ion electrode were generally performed only just before, and just after, an entire set of measurements during a toxicity test lasting 2 weeks. However, replicate measurements of Cu^{+2} activities were conducted over the test period in each test chamber in which measurements were taken. If replicate measurements uniformly varied by over 2 mV in a given diluter system, the cupric ion electrode was repolished, the inner and outer chambers of the reference electrode were refilled, and measurements were redone again. If large discrepancies between replicate measurements still existed, the electrode performance was checked in a 10^{-5} M Cu^{+2} standard.

The response time of the cupric ion electrode was dependent upon the activity of Cu^{+2} being measured but was generally less than 1.0 hours. The mV readings were taken when the chart recording indicated that mV readings were level for at least 15 minutes, or when the average mV reading from the meter did not change by greater than 0.1 mV over a 15 minute interval.

2.4 ATOMIC ABSORPTION SPECTROSCOPY

All total and dissolved copper measurements were determined by furnace atomic absorption spectroscopy with a Perkin Elmer Model 5000 atomic absorption spectrometer equipped with either a model HGA 500 graphite furnace and AS-40 auto sampler, or a model HGA 2200 graphite furnace and AS-1 auto sampler. Total copper determinations were approximated by total acid exchangeable determinations which involve only acidification prior to analyses instead of more extensive digestion procedures, which have been found to be of little or no value in analyses of Lake Superior water. Dissolved copper determinations were performed by filtering the samples through a 0.45 mu Millipore filter prior to acidification and analyses. Quality assurance was maintained with a minimum of one standard curve, two duplicate determinations, and two spike recovery determinations for every 20 samples analyzed.

2.5 FLOW-THROUGH EXPOSURE SYSTEMS

All laboratory toxicity tests other than the 96 hour static exploratory tests were flow-through tests conducted on fathead minnow larvae. Six sets of laboratory copper toxicity tests were completed. Each set of toxicity tests consisted of determining copper toxicity in four types of test water simultaneously using four diluter systems, one for each type of test water. The four diluter systems were designed and constructed to be as identical as possible (81). Each diluter system consisted of two interconnecting stainless steel headboxes, a glass diluter, randomized glass delivery tubes to exposure tanks, and 24 glass flow-through exposure chambers.

The diluent test waters used in these experiments were Lake Superior water and various types of chemically altered Lake Superior water. Filtration through 20 mesh screens, aeration, heating to 22C° and general chemical alteration (other than copper addition) in the diluent test waters were carried out in the headboxes. Chemical alterations of Lake Superior water included changes in hardness, alkalinity, pH, suspended clay and humics. Hardness was increased independently of alkalinity by pumping stock solutions of $MgCl_2$ and $CaCl_2$ into the headboxes. Alkalinities were increased independently of hardness by pumping a combination of $NaHCO_3$ and $KHCO_3$ into the headboxes. The pH was decreased independently of alkalinity by bubbling CO_2 into the headboxes. Increases in humic and suspended clay content were obtained by pumping in suspensions of Lake Superior shore red clay and solutions of humics purchased from Aldrich. Neither increases in humics nor suspended clay affected hardness or alkalinity significantly, but did increase pH 0.1-0.2 units above ambient Lake Superior water.

The diluent water flowed from the headboxes to the mixing chamber of the diluter by gravity where it was mixed with $CuSO_4$ stock that was dripped into the mixing chamber after being pumped from a stock bottle to the diluter mixing chamber with a FMI metering pump. The resultant copper solution then underwent 4 successive approximately 1:1 dilutions with diluent water such that each successive concentration was approximately 1/2 of the next highest concentration. Therefore, the lowest concentration of the five was designed to be approximately 1/16 of the highest concentration. Each diluter continuously delivered control test water and the five different concentrations of copper in the test water to 24 glass flow-through exposure chambers. Therefore, the control and each of five different copper concentrations were replicated four times within a given diluter system. Each diluter delivered solutions to the exposure chambers at approximately 15 ml/minute and maintained a volume of approximately 750 ml in each chamber corresponding to retention times of approximately 50 minutes.

Each exposure chamber in each diluter system was designated with a number indicating the relative concentration of copper in the chamber (e.g., 1 for the highest concentration to 6 for the control) and a letter (A, B, C or D) indicating which replicate delivery tube supplied the chamber from the given concentration cell in the diluter. The A and B chambers were randomly located in a row on one side of each diluter system, and the C and D chambers were randomly located on the other side of each diluter system.

2.6 FLOW-THROUGH TOXICITY TESTS

Prior to the beginning of each set of copper toxicity tests on fathead minnow larvae, eggs were obtained from the laboratory culture unit and acclimated to each type of test water for 5-6 days prior to hatching. During acclimation, the eggs were placed in screened bottom (40 mesh) 120 ml glass jars which were slowly oscillated up and down in control test waters within the control exposure chambers. The screened bottom glass jars were suspended from rocker arms which rotated at approximately 2 revolutions/minute parallel to, but above, the rows of exposure chambers. At the beginning of the copper toxicity tests, the hatched larvae were also placed in screened bottom (40 mesh) 120 ml glass jars, 10 per jar. One jar with 10 larvae in it was placed in each exposure chambers and again, slowly oscillated up and down in the control and copper test waters within the chambers.

In the flow-through copper toxicity tests described in this report, 96-hr LC50s were determined from mortality data on the 10 larvae introduced to each exposure chamber. The larvae in the A and B chambers of each diluter system were not fed, and the surviving larvae were terminated at the conclusion of the 96 hr test. The larvae in the C and D chambers were fed with brine shrimp and maintained for an additional three days after the conclusion of the 96 hr acute tests to determine the affects of copper on 7 day growth. The Trimmed Spearman-Karber computer program was used to calculate LC50 values for the unfed and fed tanks in terms of total copper, dissolved copper, and Cu^{+2} activity. At the conclusion of the 7 day growth tests, the surviving larvae were terminated, dried in an oven, and weighed. An analysis of variance was then performed on the dry weight data to determine any statistically significant (e.g., >95% probability) differences in the dry weights between larvae in control test water and larvae in test water with various levels of copper.

The hardness, alkalinity, pH, dissolved oxygen, conductivity and temperature were determined in each control test water every day during the 96 hr acute tests. In addition, the same parameters were determined at least once in the low, middle, and high copper concentration of each test water. Samples for atomic absorption spectroscopy were withdrawn from chambers representing every copper concentration in each test water at least once prior to the running of the toxicity tests, and at least twice during the 7 day test period. Duplicate sets of Cu^{+2} activity measurements were made on different days in tanks representing every copper concentration in each test water during the 7 day period, and additional sets were usually taken if large discrepancies between duplicate sets were observed. In addition, duplicate measurements, particularly in the middle concentration chambers close to the LC50, were made within the same set of measurements on a given day. In addition to the pH measurements determined with a combination electrode when general quality parameters were determined, additional pH measurements were made with a single glass electrode referenced against an Ag/AgCl double junction reference electrode in conjunction with each Cu^{+2} determination.

2.7 Cd^{+2} ELECTRODE CALIBRATION AND Cd^{+2} ACTIVITY DETERMINATIONS IN FLOW THROUGH EXPOSURE CHAMBERS

One set of laboratory flow-through cadmium tests was performed in addition to the six sets of flow-through copper toxicity tests. The set of cadmium toxicity tests was performed primarily for support of previous cadmium toxicity tests conducted at the Duluth lab. Since the subject of this report primarily concerns copper toxicity, the set of cadmium toxicity tests will not be described in detail in this report. However, the objective of the set of cadmium toxicity tests was to determine the effects of various humic levels on metal toxicity, which is identical to the objective of set 5 of the copper toxicity experiments. Therefore, the results of the cadmium toxicity tests will be briefly compared to the results of set 5 of the copper toxicity tests in Section 3.5.

The set of cadmium toxicity tests consisted of determining cadmium toxicity in four types of test water simultaneously using four diluter systems, one for each type of test water. The diluter systems used were the same ones used in copper toxicity tests, and are described in Section 2.5. The general biological and chemical procedures used for the cadmium tests were also almost identical to those described in Section 2.6 for copper. However, there are three major differences between the cadmium and copper procedures.

The first major difference involves electrode calibration. Although both the Cu^{+2} and Cd^{+2} electrodes were calibrated in .01M acetate buffer, they exhibit different behavior at low activities. The apparent non-Nernstian behavior of the Cu^{+2} electrode at low Cu^{+2} activities appears to be due to copper dissolution off the membrane and not to an actual non-Nernstian response of the electrode, as was discussed in Section 2.2. However, the Cd^{+2} electrode appears to actually exhibit non-Nernstian behavior at lower Cd^{+2} activities, as can be seen by the Cd^{+2} calibration curve presented in Figure (2-2). Even though the calibration curve is based on total rather than nominal cadmium, the calibration curve becomes non-Nernstian at cadmium activities below 3×10^{-7} M.

The non-Nernstian response of the Cd^{+2} electrode has at least two detrimental effects on the determination of Cd^{+2} activities. The determination of Cd^{+2} activities in the non-Nernstian response range of the electrode is subject to greater error than in the Nernstian range due to the decrease in the slope of the electrode response versus the log of the activity in the non-Nernstian range. Also, the limit of detection of the cadmium electrode caused

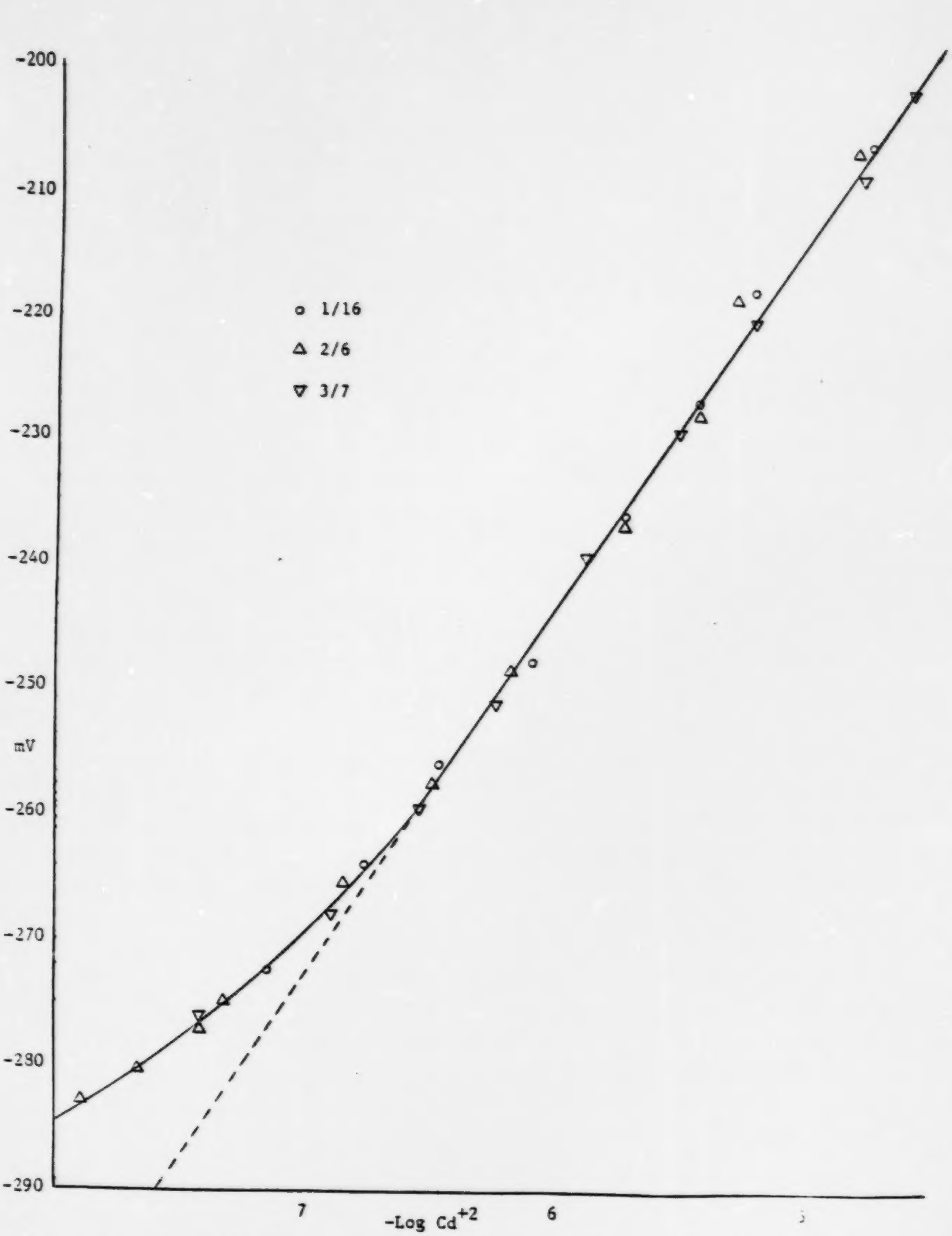

Figure 2-2. The calibration curve for the cadmium (Cd^{+2}) ion electrode

by the flattening of the calibration curve at low-activities makes the determination of some of the lower Cd^{+2} activities in some of the toxicity tests impossible. Since the non-Nernstian response of the Cd^{+2} electrode is actual instead of apparent due to any Cd^{+2} dissolution off the membrance, the use of metal buffers for calibration would probably not extend the Nernstian response range of the electrode, nor lower the limit of detection.

The second major difference between copper and cadmium procedures involves the way in which electrode readings were taken in the flow-through chambers. Although a flow-through electrode chamber was used to perform Cu^{+2} electrode readings as discussed in Section 2.3, the flow supplied by the electrode chamber was insufficient for use with the Cd^{+2} electrode. Therefore, flow was supplied to the Cd^{+2} electrode by bubbling air through the solution with the use of an air stone positioned below, and directed at, the electrode membrane surface.

The third major difference between copper and cadmium electrode procedures involves the way in which the electrode membrance surface was cleaned. Whereas the use of Orion supplied electrode polishing strips improved the performance of the Cu^{+2} electrode, it was detrimental to the performance of the Cd^{+2} electrode leading to high and often unstable mV readings. The Cd^{+2} electrode membrane surface was therefore cleaned with tissue paper.

2.8 CONNECTICUT FIELD STUDY ON THE NAUGATUCK RIVER

Static 96 hour LC50s in terms of total, dissolved, and free copper (Cu^{+2}) were determined for one day old fathead minnow larvae in water samples taken from several different sites along the Naugatuck River in Connecticut. The tests were conducted as part of a much larger study to determine the applicability of site specific criteria derived in relatively unpolluted upstream waters to site water downstream containing industrial and/or municipal sewage effluents. The Cu^{+2} LC50 determinations were made so that they could be compared to any future theoretical estimates of Cu^{+2} LC50 values if a suitable chemical speciation methodology can be developed. Since R. Carlson of the EPA lab in Duluth is preparing a report on the Connecticut field study, the study will not be described in detail in this report. However, the results of the tests in which Cu^{+2} LC50s were determined will be briefly discussed in Section 3.7.

Static tests were run in Lake Superior water for comparative purposes, water taken from a relatively unpolluted upstream site (site N1), and water taken from several downstream sites containing industrial and/or municipal sewage effluents (N4-A, N5, N6, N7). Figure 2-3 shows the location of the various sampling sites along the Naugatuck River.

The static tests were run in 1000 ml plastic test chambers in 700 ml of test water at 25°C. Duplicate test chambers, each containing 10 organisms, were used for each copper concentration and type of test water. Also, an additional test chamber containing the test water but no organisms, was set up for each copper concentration and type of test water so that electrode determinations of Cu^{+2} activities could be performed without disturbing the test organisms.

Samples for atomic absorption analyses of total and dissolved copper, and for pH, hardness, and alkalinity determinations were taken both prior to the initiation of, and after the termination of, the 96 hour acute tests. The Cu^{+2} activity for each nominal copper concentration and type of test water was determined in water from the exposure tanks without organisms within the first 24 hours of each toxicity test. Additional Cu^{+2} activities were also determined in water from the exposure chambers with organisms after the termination of the 96 hr acute toxicity tests. However, due to time limitations, Cu^{+2} activity determinations after the termination of the toxicity tests were performed only in water from exposure chambers bracketing the dissolved copper LC50 point.

The Cu^{+2} activity determinations were performed as follows. Water from a given exposure chamber was poured into a 500 ml polyethylene bottle to the top of the bottle, and then closed to the atmosphere with a screw polyethylene top into which a cupric ion selective electrode, double junction reference electrode and pH electrode had been previously tightly fitted. The sample was

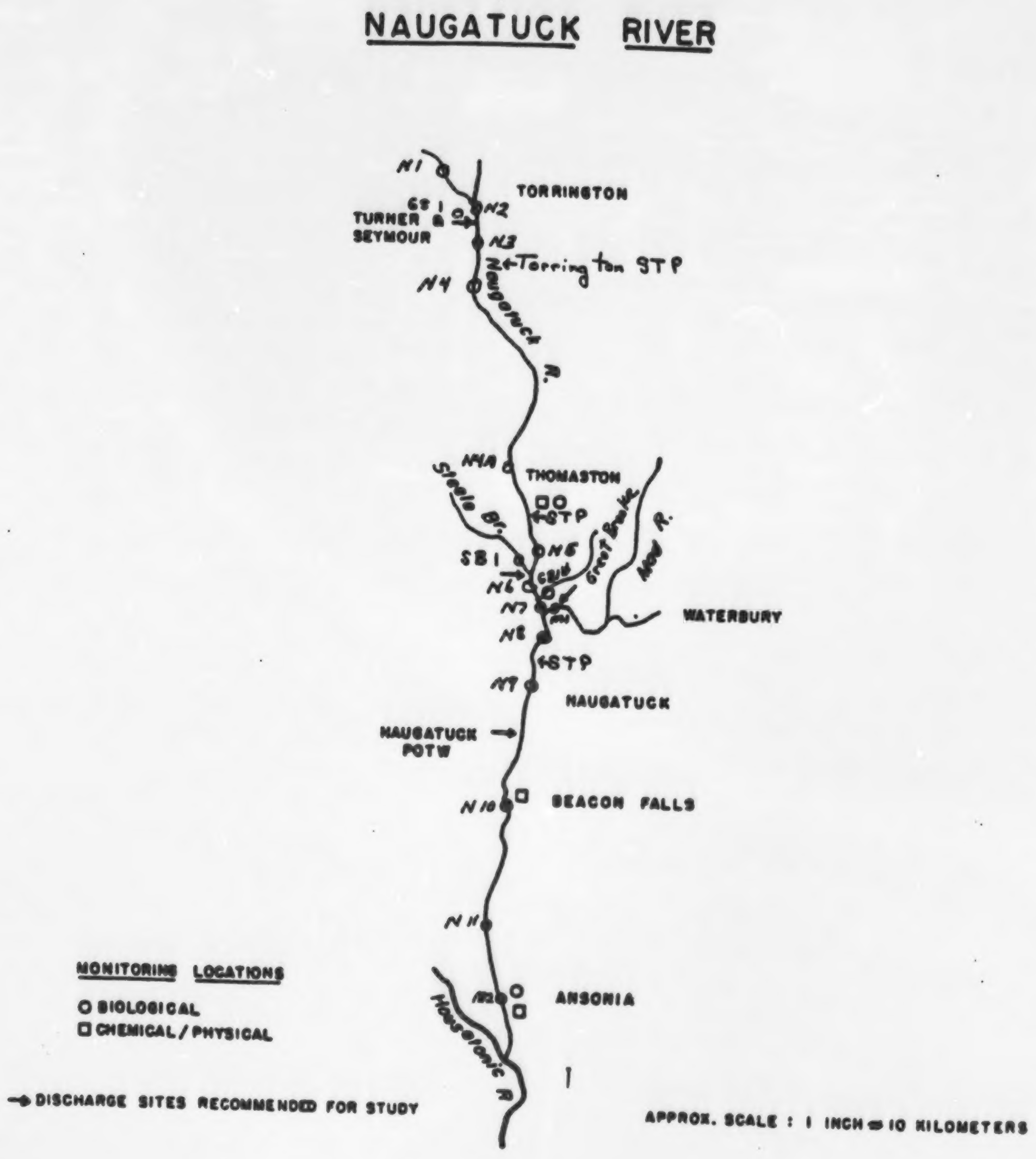
NAUGATUCK RIVER
N1
TORRINGTON
GS 1
N2
TURNER & SEYMOUR
N3
Torrington STP
N4
Naugatuck R.
N4A
THOMASTON
Steele Br.
STP
N5
SB1
N6
Great Brook
Mad R.
N7
WATERBURY
N8
STP
N9
NAUGATUCK
NAUGATUCK POTW
N10
BEACON FALLS
N11
N12
ANSONIA
Housatonic R.
MONITORING LOCATIONS
○ BIOLOGICAL
☐ CHEMICAL / PHYSICAL
→ DISCHARGE SITES RECOMMENDED FOR STUDY
APPROX. SCALE : 1 INCH = 10 KILOMETERS

Figure 2-3 Sampling sites along the Naugatuck River

closed to the atmosphere with minimal head space to prevent CO_2 exchange and the associated pH changes from occurring during the stirring required for the Cu^{+2} activity determinations. The sample was then stirred with a Teflon bar, and mV readings were recorded until they leveled off to changes of no more than 0.1mV in a 5 minute period.

2.9 STEADY STATE/EQUILIBRIUM COMPARISONS

A number of tests were performed to determine how close the steady states in the flow-through toxicity tests were to equilibrium. Tests were performed on Lake Superior water and Lake Superior water with elevated pH and alkalinity, lowered pH and alkalinity, and added humic acid. The tests were performed on test waters with nominal copper concentrations comparable to previously determined LC50 values in similar types of water.

The tests were conducted as follows. A sample was withdrawn from the flowthrough chamber for later analyses for total copper by atomic absorption analysis. The pH and Cu^{+2} activity were determined in a duplicate flow-through chamber by the same method described in section 2.3. Water was then poured from the exposure chamber into a 500 ml wide mouth Teflon bottle to the top of the bottle and then closed to the atmosphere with a screw polyethylene cap into which a Cu^{+2} ion selective electrode, and a double junction reference electrode had been previously tightly fitted. The sample was then stirred with a Teflon bar, and the mV readings from the cupric ion selective electrode were recorded every 5 minutes for the first 15 minutes, every 15 minutes for the next 45 minutes and every 30 minutes thereafter for at least 6 hours or until the readings leveled out.

At the termination of the Cu^{+2} electrode mV readings, a sample was taken from the bottle for analysis by atomic absorption for total copper. That was done so that any decrease in mV readings due to copper absorption could be differentiated from decreases due to the difference between the flow-through steady state and the closed system equilibrium. After the sample was taken, the pH of the remaining solution in the Teflon bottle was determined to make sure that the stirring during the experiment had not led to any significant

CO_2 exchange and associated pH change which would have affected the Cu^{+2} activity in addition to any differences between the flow-through steady state and the closed system equilibrium.

2.10 COPPER TITRATIONS OF LAKE SUPERIOR WATER AND RECONSTITUTED WATER

Two sets of titration experiments were performed to determine if copper-organic complexation was primarily responsible for the magnitude of the observed greater than Nernstian slopes of the cupric ion selective electrode response in Lake Superior water. The first set of experiments was designed to observe the effects of reduced organic content on the slope of the electrode response. The second set of experiments was designed to observe the effects of ionic strength, cation competition with Cu^{+2} for organic ligands, alkalinity, and pH on the slope of the electrode response.

The first set of experiments consisted of 10^{-3}M $Cu(NO_3)_2$ titrations of Lake Superior water, UV irradiated Lake Superior water, Burdick & Jackson HPLC grade water, UV irradiated Burdick & Jackson water, and Burdick & Jackson water passed through a C-18 reverse phase HPLC column. The alkalinities of the Burdick & Jackson waters were raised to that of ambient lake water by adding either Na_2CO_3 or $NaHCO_3$. The second set of experiments consisted of 10^{-3}M $CuNO_3$ titrations of Lake Superior water and seperate Lake Superior waters with the following nominal concentrations of added chemicals: $2x10^{-3}$M $Ca(NO_3)_2$, 10^{-4}M $Zn(NO_3)_2$, $6x10^{-3}$M Na NO_3, and $4x10^{-3}NaHCO_3$. Titrations of the $4x10^{-3}$M $NaHCO_3$ water were performed at pHs of 8.00 and 8.50.

The equipment and procedures used for the titrations were similar to those discussed in Sections 2.1 and 2.2 for the electrode calibrations. The major difference was that the titrations were performed primarily in solutions with a low pH buffering capacity. Changes in pH during a titration can contribute to the non-Nernstian slope, as will be discussed in Section 3.9. Since we were interested in differentiating the contribution of copper-organic complexes from that of pH changes to the non-Nerstian slope, and since the pH in equilibrium with the atmosphere can vary during the course of the titration, the titrations were performed at constant pH as follows.

The Burdick & Jackson waters to which Na_2CO_3 was added to approximate ambient lake water alkalinity and lake waters with added $NaHCO_3$ were bubbled with CO_2 until the pH of the solution was below 8.00. The ambient lake water and other chemically altered lake water already had pHs below 8.00 due to supersaturation with CO_2. If the pH of the solution was below 7.95, it was then bubbled with air until the pH was 7.95 and then stirred until the pH was 8.00. When the pH was 8.00, the mV reading related to the Cu^{+2} activity in the blank was taken, a sample was withdrawn for atomic absorption of total copper, and an aliquot of 10^{-3}M $CuNO_3$ was added. The addition of the $CuNO_3$ would drop the pH below 8.00 again. After stirring slowly brought the pH back to 8.00, another mV reading was taken, another sample was withdrawn for total copper analyses, and another 10^{-3}M $CuNO_3$ addition was made. The same procedure was repeated until the titration was terminated. All of the constant pH titrations were performed at a pH of 8.00 except one at a pH of 8.50.

2.11 CHEMICAL SPECIATION CALCULATIONS

The chemical speciation calculations in this report are based primarily on the substitution of Cu^{+2} LC50s, derived from cupric ion selective determinations of Cu^{+2} activities, into equations (1-1 to 1-7). In addition, for comparative purposes, the calculations were also performed by the input of dissolved copper LC50s into the REDEQL chemical equilibrium program. The REDEQL program was also used to model the effect of the amino acid glycine on chemical speciation.

All equilibrium calculations of $CuOH^+$, $Cu(OH)_2^\circ$, $CuCO_3^\circ$ and $Cu(CO_3)_2^{-2}$ in this report, including those using REDEQL, were performed using the stability constant values experimentally determined by Sunda and Hansen (35) with a cupric ion selective electrode. The stability constants are for 25°C and zero (by correction) ionic strength. No standard enthalpy of formation values for the copper hydroxy or carbonate complexes could be found in the literature, so temperature corrections of the stability constants to the experimental temperature in the flow-through systems of 22°C were not possible. However, approximate values of the ion product of water K_w and the acid dissociation constant K_{a2} for HCO_3^- going to CO_3^{-2} at 22°C were used in the calculations based on graphical interpolation between values at several other temperatures.

The stability constants of formation reported by Sunda and Hansen (35) for $CuOH^+$, $CuCO_3^{\circ}$ and $Cu(CO_3)_2^{-2}$ are very close to those listed in Martel and Smith (58). However, there are major disagreements in the literature over the magnitude of the stability constant for the formation of $Cu(OH)_2^{\circ}$ with reported values differing by as much s 10^4(35). Although there have been several methods used to determine the $Cu(OH)_2^{\circ}$ stability constant, the method using the cupric ion selective electrode appears to be the most direct.

There have been three values of the $Cu(OH)_2^{\circ}$ stability constant determined with a cupric ion selective electrode (35,66,81), all of which have been reported subsequent to the most recent listings of stability constants by Martel and Smith (58). The value of the $Cu(OH)_2^{\circ}$ stability constant reported by Sunda and Hanson (35) is very close to that reported by Paulson (81), but is more than three orders of magnitude less than that reported by Vuceta and Morgan (66). We have decided to use the value of the stability constant reported by Sunda and Hanson (35) for the following reasons:

1. The reported value is close to that reported by Paulson (81).
2. Both Paulson (81) and Sunda and Hanson (35) corrected for copper absorption whereas Vuceta and Morgan (66) did not.
3. The agreement between our electrode determination of Cu^{+2} activities and REDEQL predicted Cu^{+2} activities in systems with higher than ambient lake water alkalinity and/or pH is greater using the constant reported by Sunda and Hanson than that using the constant originally listed in the REDEQL data base or the constant reported by Vuceta and Morgan (66).

The activity coefficient values used in equations (1-1 to 1-6) were determined from the substitution of the estimated ionic strength for each test water into the Davies equation (1-8). Estimates of the ionic strength of each test water were made from the following equation based on the assumption that most of the alkalinity of natural freshwaters below a pH of 8.5 is due to HCO_3^-:

$$I = (2\text{x}10^{-5})\ (\text{Hardness in mg/L as } CaCO_3) + 1/2\ (\text{Alkalinity in eq/L}) + 1/2(7\text{x}10^{-5}) + 1/2(2\text{x}10^{-4}) + 1/2\sum_i C_i Z_i^2$$

where

I = estimated ionic strength

(7×10^{-5}) = sum of the average concentrations of Na^+ and K^+ in Lake Superior water (57).

(2×10^{-4}) = sum of the average concentrations of Cl^- and NO_3^- and four times the average concentration of SO_4^{-2} in Lake Superior water (57).

$\sum_i C_i Z_i^2$ = sum of the product of the concentration of any added cations and anions above the average concentration in ambient Lake Superior water times the ion charge squared.

3. RESULTS AND DISCUSSION

Six sets of flow-through copper toxicity tests, one set of flow-through cadmium toxicity tests, and a field study on the Naugatuck River in Connecticut were completed. Each set of flow-through toxicity tests consisted of 96 hr LC50 determinations and 7 day growth studies in four types of test water. The results of the LC50 determinations and related speciation calculations for the unfed tanks are summarized for every test water in each set in Tables 3-1, 3-2, and 3-3. The results of the one set of Cd^{+2} toxicity tests are compared to those of set 5 of the Cu^{+2} toxicity tests in Section 3.5.

The LC50 determinations for the fed tanks in terms of total, dissolved and free copper (Cu^{+2}) were very close to that of the unfed tanks, which indicates that the absorption of copper onto the food and the effects of feeding were probably negligible. However, because most of the Cu^{+2} determinations were performed in the unfed tanks and because feeding is not a standard procedure for 96 hr acute tests, the chemical speciation calculations are based on the unfed tanks. The LC50 values in terms of total, dissolved, and free copper (Cu^{+2}) for the static field 96 hour acute tests performed on samples from the Naugatuck River are presented in Table 3-4. The sites along the Naugatuck River from which samples were taken were shown previously in figure 2-3.

Table 3-1 lists for each test water in each set, by column, any chemical constituent added to ambient Lake Superior water, the average pH, alkalinity, and hardness of the test water, and the LC50 determinations for the test water in terms of total copper, dissolved copper, Cu^{+2} activity and Cu^{+2} concentration. The table lists two mean pH values for each test water. The first value in each case is the mean pH determined with a combination pH electrode that was calibrated in one buffer at pH 7. The second value in each case is the mean pH value determined with a single glass pH electrode (Fisher or Orion) referenced against a double junction Ag/AgCl electrode and calibrated using two buffers (pH 7 and pH 10). The discrepancies between mean pH values are usually < 0.2 pH units, but are large enough to significantly affect some of the speciation calculations at higher pHs as shown in Tables 3-2 and 3-3.

Table 3-2 lists for each test water in each set, by column, any chemical constituent added to the ambient Lake Superior water, the mean pH of the test water, the LC50 in terms of dissolved Cu in moles/L, the LC50 value in terms of the Cu^{+2} concentration in moles/L and the corresponding calculated concentrations of $CuOH^{+}$, $Cu(OH)_2^{\circ}$, $CuCO_3^{\circ}$, $Cu(CO_3)_2^{-2}$, and Cu-Org in moles/L where Cu-Org stands for a sum of the concentrations of all theoretical copperorganic or other unknown complexes. The calculated concentrations of the inorganic copper species were determined by substituting in the experimentally determined pH, alkalinity, and Cu^{+2} activity at the LC50 point into equations (1-1) through (1-7). The sum of theoretical copper-organic or other unknown complexes represented by Cu-Org was calculated from the difference between the dissolved copper and the sum of all major inorganic copper species at the LC50 point. A value of zero means that the sum of all major inorganic species was equal to or greater than the dissolved copper. Table 3-2 lists three sets of calculated concentrations for each test water, one from substituting the combination electrode mean pH into equations (1-2) to (1-7), one from substituting in the single glass electrode mean pH values, and one from substituting in the average of the two mean pH values.

Table 3-3 lists for each test water in each set, by column, any chemical constituent added to the ambient Lake Superior water to form the test water, the mean pH of the test water, the LC50 in terms of dissolved copper in mol/L, the calculated percentages of dissolved copper at the LC50 point contributed by Cu^{+2}, $CuOH^{+}$, $Cu(OH)_2^{\circ}$, $CuCO_3^{\circ}$, and $Cu(CO_3)_2^{-2}$, respectively, the sum of those inorganic percentages, and the possible Cu-Org percentage calculated from the difference between 100% and the sum of inorganic percentages. Again, as in Table 3-2, there are three sets of data for each test water corresponding to calculations based on pH values determined with the combination pH electrode, pH values determined with the single glass electrode, and averages of the mean combination and single glass electrode pH values.

A discussion of the results of the six sets of copper toxicity tests concluded is given below. Unless otherwise stated, all discussion concerning chemical speciation will be based on calculations which used the average of the combination pH electrode and single glass electrode mean pH values (e.g., the bottom pH for each test water in Tables 3-2 and 3-3).

TABLE 3-1. General water quality characteristics and 96 hr LC50 values in terms of total copper, dissolved copper, Cu^{+2} activity and Cu^{+2} concentration for the test waters of each set of copper toxicity experiments. The parentheses show 95% confidence intervals.

	pH							
	Comb. Elect.	Electr. vs Ref.	Alk. mg/L	Hard. mg/L	LC50 Tot. Cu ug/L	LC50 Diss. Cu ug/L	LC50 (Cu^{+2}) ug/L	LC50 $[Cu^{+2}]$ ug/L
Set 1 -- 4/83 (Ca^{+2}, Mg^{+2}, Na^{+} Affects)								
Ambient Hardness	8.10	8.16	42.6	47.0	32.0 (25-41)	28.0 (22-36)	0.48 (0.3-0.7)	0.57
2×10^{-3} M $CaCl_2$	8.01	8.09	43.1	243	119 (89-166)	109 (82-152)	3.64 (2.5-5.2)	5.42
2×10^{-3} $MgCl_2$	8.01	8.10	43.3	255	48.0 (37-63)	40.0 (31-53)	1.13 (0.8-1.6)	1.64
2×10^{-3} NaCl	8.10	8.14	43.3	47.2	75.0 (50-111)	70.0 (47-104)	2.85 (2.0-4.0)	3.70
Set 2 -- 6/83 (pH Effects at Ambient Alkalinity)								
CO_2 Bubbling	6.63	6.53	48.9	49.2	8.0 (6.9-8.5)	7.0 (6.0-7.4)	0.79 (0.6-1.1)	0.95
CO_2 Bubbling	7.30	7.40	44.5	46.2	23.0 (18-29)	19.0 (15-24)	1.60 (1.1-2.2)	1.90
Ambient pH	8.02	8.10	42.8	45.1	60.0 (42-86)	50.0 (35-72)	1.67 (1.0-2.1)	1.99
NaOH Addition	8.65	8.81	47.4	45.2	82.0 (70-120)	71.0 (61-104)	0.45 (0.3-0.6)	0.56
Set 3 -- 7/83 (pH effects at 3X Ambient Alkalinity)								
(K^{+}, Na^{+}) HCO_3, CO_2	7.14	7.16	155	46.2	22.0 (17-28)	19.0 (15-24)	1.64 (1.3-2.1)	2.14

Table 3-1 (continued)

	pH							
	Comb. Elect.	Electr. vs Ref.	Alk. mg/L	Hard. mg/L	LC50 Tot. Cu ug/L	LC50 Diss. Cu ug/L	LC50 (Cu^{+2}) ug/L	LC50 $[Cu^{+2}]$ ug/L
$(K^+, Na) HCO_3, CO_2$	7.90	7.99	148	45.0	79.0 (53-116)	56.0 (45-98)	1.00 (0.7-1.5)	1.30
$(K^+, Na) HCO_3, CO_2$	8.50	8.64	150	45.0	157 (99-249)	136 (86-216)	0.61 (0.4-1.0)	0.79
HCl Added to Lower pH and Alkalinity	7.16	7.17	26.4	45.0	22.0 (16-31)	21.0 (15-30)	3.64 (2.4-5.5)	4.26
Set 4 -- 10/83 (Effects of Clay, Humics)								
Ambient Lake Water	7.93	8.15	40.8	42.5	105 (69-163)	87.0 (57-135)	2.75 (2.0-4.3)	3.25
Clay Added (70 NTU)	8.12	8.36	46.5	48.0	149 (117-191)	66.0 (52-84)	1.11 (0.9-1.4)	1.33
Humics (5 mg/L TOC)	7.91	8.10	44.2	45.5	289 (229-367)	249 (197-317)	1.04 (0.8-1.5)	1.24
Clay and Humics Added	8.17	8.28	48.5	46.8	430 (313-593)	254 (185-350)	0.96 (0.7-1.5)	1.14
Set 5 -- 11/83 (Effects of Variable Humic Concentrations)								
Ambient Lake Water	7.94	8.12	43.0	45.0	83.0 (64-107)	74.0 (57-96)	2.68 (2.1-3.5)	3.18
Humics (1.25 mg/L TOC)	7.91	8.11	42.2	45.0	132 (113-153)	102 (87-117)	1.88 (1.6-2.3)	2.23
Humics (2.5 mg/L TOC)	7.94	8.06	43.8	45.5	244 (208-326)	198 (169-264)	1.88 (1.5-2.9)	2.24
Humics (5.0 mg/L TOC)	7.95	8.11	42.8	45.0	298 (238-373)	245 (196-306)	0.96 (0.8-1.5)	1.14

Table 3-1 (continued)

	pH							
	Comb. Elect.	Electr. vs Ref.	Alk. mg/L	Hard. mg/L	LC50 Tot. Cu ug/L	LC50 Diss. Cu ug/L	LC50 (Cu^{+2}) ug/L	LC50 $[Cu^{+2}]$ ug/L
Set 6 -- 5/84 (Effects of Variable Alkalinities)								
HCL Added, CO_2 reduced	7.84	7.84	17.0	44.0	68.0 (58-80)	46.0 (30-54)	4.51 (3.68-5.52)	5.27
Ambient Lake Water	7.87	7.99	42.0	44.0	95.0 (75-119)	79.0 (62-99)	3.33 (2.54-4.36)	3.95
(K^+, Na^+) HCO_3, CO_2	7.92	8.04	161	44.0	51.0 (32-83)	39.0 (25-64)	0.51 (0.33-0.80)	0.67
(K^+, Na^+) HCO_3, CO_2	7.96	8.04	318	45.0	66.0 (57-77)	46.0 (40-54)	0.29 (0.23-0.34)	0.42

Table 3-2. The average pH, 96 hr LC50 in terms of dissolved copper (moles/L), 96 hr LC50 in terms of Cu^{+2} concentration (moles/L), and corresponding calculated concentrations (moles/L) of $CuOH^+$, $Cu(OH)_2$, $CuCO_3$, $Cu(CO_3)_2^{-2}$ and Cu-Org for the test waters of each set of copper toxicity experiments.

	pH^a	LC50 Diss. Cu $\times 10^9$ M^{-1}	LC50 $[Cu^{+2}]$ $\times 10^9$ M^{-1}	LC50 $[CuOH^+]$ $\times 10^9$ M^{-1}	LC50 $[Cu(OH)_2°]$ $\times 10^9$ M^{-1}	LC50 $[CuCO_3°]$ $\times 10^9$ M^{-1}	LC50 $[Cu(CO_3)_2^{-2}]$ $\times 10^9$ M^{-1}	LC50 [Cu-Org] $\times 10^9$ M^{-1}
Set 1 -- 4/83 (Ca^{+2}, Mg^{+2}, Na^+ Effects)								
Ambient Hardness	8.10	441	7.55	23.9	4.61	180	2.92	221
	8.16	441	7.55	27.4	6.07	206	3.84	189
	8.13	441	7.55	25.5	5.26	193	3.36	205
2×10^{-3} M $CaCl_2$	8.01	1720	85.3	155	23.1	1070	17.1	368
	8.09	1720	85.3	186	33.4	1290	24.7	98.2
	8.05	1720	85.3	168	27.5	1170	20.4	248
2×10^{-3} M $MgCl_2$	8.01	630	25.8	48.1	7.18	335	5.25	208
	8.10	630	25.8	59.1	10.9	411	7.92	115
	8.05	630	25.8	53.0	8.73	368	6.36	168
2×10^{-3} M NaCl	8.10	1110	58.3	145	27.4	1060	18.8	0^b
	8.14	1110	58.3	160	33.0	1160	22.5	0b
	8.12	1110	58.3	152	30.0	1110	20.5	0b
Set 2 -- 6/83 (pH Effects at Ambient Alkalinity)								
CO_2 Bubbling	6.63	110	12.4	1.34	0.0087	10.2	0.0057	83.6
	6.53	110	12.4	1.06	0.0055	8.05	0.0036	86.0
	6.58	110	12.4	1.18	0.0068	8.87	0.0044	85.0
CO_2 Bubbling	7.30	299	30.0	12.6	0.386	101	0.279	155
	7.40	299	30.0	16.0	0.612	127	0.436	125
	7.35	299	30.0	14.1	0.479	111	0.334	143
Ambient pH	8.02	787	31.3	69.3	11.1	525	7.18	14.2
	8.10	787	31.3	83.3	16.1	631	10.3	14.6
	8.06	787	31.3	75.6	13.2	567	8.38	91.3

Table 3-2 (continued)

	pH^a	LC50 Diss. Cu x 10^9 M^{-1}	LC50 [Cu^{+2}] x 10^9 M^{-1}	LC50 [$CuOH^+$] x 10^9 M^{-1}	LC50 [$Cu(OH)_2°$] x 10^9 M^{-1}	LC50 [$CuCO_3°$] x 10^9 M^{-1}	LC50 [$Cu(CO_3)_2^{-2}$] x 10^9 M^{-1}	LC50 [Cu-Org] x 10^9 M^{-1}
NaOH Added	8.65	1120	7.08	79.6	54.3	646	40.5	291
	8.81	1120	7.08	115	113	915	81.0	0b
	8.72	1120	7.08	93.9	75.5	755	55.4	131
Set 3 -- 7/83 (pH Effects at 3X Ambient Alkalinity)								
$KHCO_3$ and $NaHCO_3$	7.14	299	33.6	9.17	0.189	243	1.71	11.3
Added, CO_2	7.16	299	33.6	9.59	0.207	254	1.88	0.00^c
Bubbling	7.15	299	33.6	9.37	0.198	248	1.79	6.03
$KHCO_3$ and $NaHCO_3$	7.90	1050	15.7	32.1	3.81	805	30.8	160
Added, CO_2	7.99	1050	15.7	39.6	5.81	993	46.9	0^a
Bubbling	7.94	1050	15.7	35.4	4.65	889	37.7	62.8
$KHCO_3$ and $NaHCO_3$	8.50	2140	12.5	78.1	37.0	1940	294	0^b
Added, CO_2	8.64	2140	12.5	108	70.5	2650	547	0^b
Bubbling	8.56	2140	12.5	90.5	49.6	2240	390	0^b
HCl Added	7.16	331	67.4	20.8	0.461	98.3	0.114	144
to Lower pH	7.17	331	67.4	21.3	0.483	101	0.120	141
and Alkalinity	7.16	331	67.4	21.0	0.471	99.6	0.117	142
Set 4 -- 10/83 (Effects of Humics and Suspended Clay)								
Ambient Lake	7.93	1370	51.2	93.0	12.2	676	7.20	530
Superior Water	8.15	1370	51.2	154	33.3	1110	19.3	0.00^c
	8.03	1370	51.2	116	18.9	838	11.1	330
Clay Added	8.12	1040	20.9	58.1	11.7	475	8.8	457
to Turbidity	8.36	1040	20.9	101	35.3	816	26.2	39.5
of 70 NTU	8.22	1040	20.9	73.8	18.8	603	14.2	309

Table 3-2 (continued)

	pH^a	LC50 Diss. Cu $\times 10^9$ M^{-1}	LC50 $[Cu^{+2}]$ $\times 10^9$ M^{-1}	LC50 $[CuOH^+]$ $\times 10^9$ M^{-1}	LC50 $[Cu(OH)_2°]$ $\times 10^9$ M^{-1}	LC50 $[CuCO_3°]$ $\times 10^9$ M^{-1}	LC50 $[Cu(CO_3)_2^{-2}]$ $\times 10^9$ M^{-1}	LC50 [Cu-Org] $\times 10^9$ M^{-1}
Humics Added	7.91	3920	19.5	33.6	4.17	262	2.88	3630
(5 mg/L TOC)	8.10	3920	19.5	52.0	10.0	406	6.89	3430
	8.00	3920	19.5	40.9	6.19	319	4.27	3540
Humics and Clay	8.17	3860	18.1	56.3	12.7	481	10.5	3280
Added (5 mg/L	8.28	3860	18.1	72.5	21.1	617	17.3	3120
TOC, 70 NTU)	8.22	3860	18.1	63.3	16.1	538	13.2	3210
Set 5 -- (Variable Humic Concentrations)								
Ambient Lake	7.94	1160	50.1	92.3	12.3	703	8.00	293
Superior Water	8.12	1160	50.1	140	28.2	1060	18.3	0^a
	8.02	1160	50.1	112	17.8	847	11.6	131
Humics Added	7.91	1610	35.2	60.6	7.53	454	4.76	1050
(1.25 mg/L	8.11	1610	35.2	95.9	18.9	713	22.7	736
TOC)	8.00	1610	35.2	74.5	11.4	557	7.15	924
Humics Added	7.94	3120	35.2	64.8	8.62	503	5.86	2500
(2.50 mg/L	8.06	3120	35.2	85.6	15.0	661	10.1	2310
TOC)	8.00	3120	35.2	73.8	11.2	571	7.56	2420
Humics Added	7.95	3860	18.0	33.9	4.63	257	2.98	3540
(5.0 mg/L	8.11	3860	18.0	49.1	9.65	369	6.16	3410
TOC)	8.02	3860	18.0	40.1	6.47	303	4.16	3490
Set 6 -- 5/84 (Alkalinity Effects at Ambient pH)								
HCL Added,	7.84	724	82.9	123	13.0	374	1.32	130
CO_2 Reduced	7.84	724	82.9	123	13.0	374	1.32	130
	7.84	724	82.9	123	13.0	374	1.32	130

Table 3-2 (continued)

	pH^a	LC50 Diss. Cu $\times 10^9$ M^{-1}	LC50 $[Cu^{+2}]$ $\times 10^9$ M^{-1}	LC50 $[CuOH^+]$ $\times 10^9$ M^{-1}	LC50 $[Cu(OH)_2°]$ $\times 10^9$ M^{-1}	LC50 $[CuCO_3°]$ $\times 10^9$ M^{-1}	LC50 $[Cu(CO_3)_2^{-2}]$ $\times 10^9$ M^{-1}	LC50 [Cu-Org] $\times 10^9$ M^{-1}
Ambient Lake	7.87	1240	62.2	97.7	11.1	729	6.93	333
Water	7.99	1240	62.2	129	19.4	965	12.1	52.1
	7.93	1240	62.2	111	14.2	827	8.91	217
$(K^+, Na^+)HCO3, CO_2$	7.96	724	6.54	10.9	1.45	562	57.1	86.2
Added to	8.04	724	6.54	13.2	2.11	677	82.9	0^a
6x Ambient	8.00	724	6.54	11.9	1.72	609	67.1	27.5
Alkalinity								

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[a] For each test water, the top pH listed is the average pH value determined with a combination pH electrode that was calibrated in one buffer at pH 7. The middle pH is the average pH value determined with a single glass electrode referenced against a double junction Ag/AgCl reference electrode and calibrated in two buffers (pH 7 and pH 10). The bottom pH is the average of the top pH and middle pH.

[b] The sum of calculated concentrations of all major inorganic species exceeded the dissolved copper.

[c] The sum of calculated concentrations of all major inorganic species equaled the dissolved copper.

Table 3-3. The average pH, 96 hr LC50 in terms of dissolved copper (moles/L), percentages of dissolved copper made up by Cu^{+2}, $CuOH^{+}$, $Cu(OH)_2^{\circ}$, $CuCO_3^{\circ}$, $Cu(CO_3)_2^{\circ}$, respectively, the sum of the inorganic percentages, and the theoretical copper organic percentages

	pH[a]	LC50 Cu Diss. $\times 10^9$ M^{-1}	% Cu^{+2}	% $CuOH^{+}$	% $Cu(OH)_2^{\circ}$	% $CuCO_3^{\circ}$	% $Cu(CO_3)_2^{-2}$	% Inorganic	Cu-Org
Set 1 -- 4/83 (Ca^+, Mg_+, Na^+ Effects)									
Ambient Lake Superior Water	8.10	441	2.08	5.42	1.05	40.8	0.662	49.9	50.1
	8.16	441	2.03	6.21	1.38	46.7	0.871	57.1	42.9
	8.13	441	2.03	5.78	1.19	43.8	0.762	53.5	46.5
2×10^{-3} M $CaCl_2$ Added	8.01	1720	4.96	9.01	1.34	62.2	0.994	78.6	21.4
	8.09	1720	4.96	10.8	1.94	75.0	1.44	94.3	5.71
	8.05	1720	4.96	9.77	1.60	68.0	1.19	85.6	14.4
2×10^{-3} M $MgCl_2$ Added	8.01	630	4.10	7.63	1.14	53.2	0.833	66.9	33.1
	8.10	630	4.10	9.38	1.73	65.2	1.26	81.7	18.3
	8.05	630	4.10	8.41	1.39	58.4	1.01	73.3	26.7
2×10^{-3} M NaCl Added	8.10	1100	5.30	13.2	2.49	96.4	1.71	119	0[b]
	8.14	1100	5.30	14.5	3.00	105	2.05	131	0[b]
	8.12	1100	5.30	13.8	2.73	101	1.86	125	0[b]
Set 2 -- 6/83 (pH Effects at Ambient Alkalinity)									
CO_2 Bubbling	6.63	110	13.5	1.22	0.0079	9.27	0.005	24.0	76.0
	6.53	110	13.5	0.964	0.0050	7.32	0.003	21.8	78.2
	6.58	110	13.5	1.07	0.0062	8.06	0.004	22.7	77.3
CO_2 Bubbling	7.30	299	10.0	4.21	0.129	33.8	0.093	48.3	51.7
	7.40	299	10.0	5.35	0.205	42.5	0.146	58.2	41.8
	7.35	299	10.0	4.72	0.160	37.1	0.112	52.1	47.9

Table 3-3 (continued)

	pH[a]	LC50 Cu Diss. x 10^9 M^{-1}	% Cu^{+2}	% $CuOH^+$	% $Cu(OH)_2^\circ$	% $CuCO_3^\circ$	% $Cu(CO_3)_2^{-2}$	% Inorganic	% Cu-Org
Ambient pH	8.02	787	3.98	8.81	1.41	66.7	0.912	81.9	18.1
	8.10	787	3.98	10.6	2.05	80.2	1.1	98.2	1.85
	8.06	787	3.98	9.61	1.68	72.0	1.07	88.4	11.6
NaOH Added	8.65	1120	0.753	7.11	4.85	57.7	3.62	74.0	26.0
	8.81	1120	0.753	10.3	10.1	81.7	7.23	110	0[b]
	8.72	1120	0.753	8.38	6.74	67.4	4.95	88.3	11.7
Set 3 -- 7/83 (pH Effects at 3X Ambient Alkalinity)									
$KHCO_3$ and	7.14	299	11.2	3.07	0.063	81.3	0.571	96.2	3.79
$NaHCO_3$ Added	7.16	299	11.2	3.21	0.069	84.9	0.629	100	0.00[c]
CO_2 Bubbling	7.15	299	11.2	3.13	0.066	82.9	0.599	98.0	2.02
$KHCO_3$ and	7.90	1050	1.94	3.06	0.362	76.7	2.93	84.8	15.2
$NaHCO_3$ Added	7.99	1050	1.94	3.77	0.553	94.6	4.47	105	0[b]
CO_2 Bubbling	7.94	1050	1.94	3.37	0.443	84.7	3.59	94.0	5.98
$KHCO_3$ and	8.50	2140	0.583	3.65	1.73	90.7	13.7	110	0[b]
$NaHCO_3$ Added	8.64	2140	0.583	5.05	3.29	124	25.6	158	0[b]
	8.56	2140	0.583	4.23	2.32	105	18.2	130	0[b]
HCl Added to	7.16	331	20.4	6.28	0.139	29.7	0.034	56.5	43.5
Lower pH and	7.17	331	20.4	6.44	0.146	30.5	0.036	57.5	42.5
Alkalinity	7.164	331	20.4	6.34	0.142	30.1	0.035	57.0	43.0
Set 4 -- 10/83 (Effects of Humics and Suspended Clays)									
Ambient Lake	7.93	1370	3.74	6.79	9.891	49.3	0.526	61.3	38.7
Superior Water	8.15	1370	3.74	11.2	2.43	81.0	1.41	100	0.00[c]
	8.03	1380	3.74	8.47	1.38	61.2	0.810	75.9	24.1

Table 3-3 (continued)

	pH^a	LC50 Cu Diss. x 10^9 M^{-1}	% Cu^{+2}	% $CuOH^+$	% $Cu(OH)_2^\circ$	% $CuCO_3^\circ$	% $Cu(CO_3)_2^{-2}$	% Inorganic	% Cu-Org
Clay Added to 70 NTU	8.12	1040	2.01	5.59	1.13	45.7	0.850	56.1	43.9
	8.36	1040	2.01	9.71	3.39	78.5	2.52	96.2	3.8
	8.22	1040	2.01	7.10	1.81	58.0	1.37	70.3	29.7
Humics Added (5 mg/L TOC)	7.91	3920	0.497	0.857	0.106	6.68	0.073	7.37	92.6
	8.10	3920	0.497	1.33	0.255	10.4	0.176	12.6	87.4
	8.00	3920	0.497	1.04	0.158	8.14	0.109	9.95	90.1
Clay and Humics Added (70 NTU, (5 mg/L TOC)	8.17	3860	0.469	1.46	0.329	12.5	0.272	15.0	85.0
	8.28	3860	0.469	1.88	0.547	16.0	0.448	19.3	80.7
	8.22	3860	0.469	1.64	0.417	13.9	9.342	16.8	83.2
Set 5 -- 11/83 (Effects of Variable Humics Concentrations									
Ambient Lake Superior Water	7.94	1160	4.32	7.96	1.06	60.6	0.690	74.7	25.3
	8.12	1160	4.32	12.1	2.43	91.4	1.58	112	0^a
	8.02	1160	4.32	9.66	1.53	73.0	1.00	89.7	11.3
Humics Added (1.25 mg/L	7.91	1610	2.19	3.76	0.468	28.2	0.296	34.9	65.1
	8.11	1610	2.19	5.96	1.17	44.3	0.727	54.3	45.7
	8.00	3120	1.13	2.37	0.359	18.3	0.242	22.4	77.6
Humics Added (2.50 mg/L	7.94	3120	1.13	2.08	0.276	16.1	0.188	19.8	80.2
	8.06	3120	1.13	2.74	0.481	21.2	0.324	25.9	74.1
	8.00	3120	1.13	2.37	0.359	18.3	0.242	22.4	77.6
Humics Added (5.0 mg/L TOC)	7.95	3860	0.466	0.878	0.120	6.66	0.077	8.21	91.8
	8.11	3860	0.466	1.27	0.250	0.56	0.159	11.7	88.3
	8.02	3860	0.466	1.04	0.168	7.85	0.108	9.64	90.4

Table 3-3 (continued)

	pH[a]	LC50 Cu Diss. $\times 10^{9}$ M^{-1}	% Cu^{+2}	% $CuOH^{+}$	% $Cu(OH)_2^{\circ}$	% $CuCO_3^{\circ}$	% $Cu(CO_3)_2^{-2}$	% Inorganic	% Cu-Org
Set 6 -- 5/84 (Alkalinity Effects at Ambient pH)									
HCL Added to	7.84	724	11.5	16.9	1.80	51.6	0.182	82.0	18.0
reduce alkalinity	7.84	724	11.5	16.9	1.80	51.6	0.182	82.0	18.0
to below ambient, and CO_2 removed to maintain ambient pH	7.84	724	11.5	16.9	1.80	51.6	0.182	82.0	18.0
Ambient Lake	7.87	1240	5.02	7.88	0.895	58.3	0.559	73.1	26.9
H_2O	7.99	1240	5.02	10.4	1.56	77.8	0.976	95.8	4.2
	7.93	1240	5.02	8.95	1.15	66.7	0.719	82.5	17.5
$(K^+, Na^+)HCO_3$, CO_2	7.92	614	1.71	2.79	0.350	76.2	3.09	84.4	15.6
Added to 3x	8.04	614	1.71	3.68	0.606	101	5.43	113	0[a]
Ambient Alkalinity	8.00	614	1.71	3.18	0.448	86.3	4.04	95.9	4.1
$(K^+, Na^+)HCO_2$, CO_2	7.96	724	0.904	1.51	0.200	77.6	7.88	8.1	11.9
Added to 6x	8.04	724	0.904	1.82	0.291	93.5	11.4	108	0[a]
Ambient Alkalinity	8.00	724	0.904	1.64	0.238	84.1	9.27	96.2	3.8

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[a] For each test water, the top pH listed is teh average pH value determined with a combination pH electrode that was calibrated in one buffer at pH 7. The middle pH is the average pH value determined with a single glass electrode referenced against an Ag/AgCl double junction reference electrode and calibrated in two buffers (pH 7 and pH 10). The bottom pH is the average of the top pH and middle pH value.

[b] The sum of calculated major inorganic percentages exceeded 100% of dissolved copper.

[c] The sum of calculated major inorganic percentages was equal to 100% of dissolved copper.

There will, in some cases, be given several different interpretations of the data dependent upon whether or not a theoretical copper-organic fraction is assumed to be present. The steeper than expected non-Nernstian electrode response slopes, the associated large differences between dissolved copper and the calculated sum of all known major inorganic complexes in some test waters, and the relatively high stability constants for the formation of many copper organic complexes has led to our speculation that there may be a substantial copper-organic fraction and/or unknown copper-inorganic fraction in test waters with ambient or lower lake water pH and/or alkalinity as will be discussed in greater detail in Section 3.10. Despite substantial evidence for the presence of a significant copper-organic fraction in some test waters, the calculated concentration of the fraction and the proportion of dissolved copper it represents may be subject to large error since the calculations depend upon the difference between dissolved copper and the sum of the calculated concentrations of all known major copper-inorganic complexes. The reason is that each of the calculated concentrations of major inorganic copper species may be subject to substantial error due to errors in the Cu^{+2} and pH determinations and errors in the stability constants used in the calculations. The possible large errors in the calculations of the concentrations and proportions of theoretical copper-organic complexes along with the uncertainty in their existence should be kept in mind in reading the discussion of the six sets of copper toxicity tests given below.

3.1 SET 1 - SEPARATE EFFECTS OF $MgCl_2$, $CaCl_2$, AND NaCl ADDITIONS ON COPPER TOXICITY IN LAKE SUPERIOR WATER

The main objective of the first set of copper toxicity tests was to determine the relative effects of the two principal components of hardness, Ca^{+2} and Mg^{+2}, on copper toxicity when added separately to Lake Superior water. In addition, the effect of adding Na^{+} on copper toxicity in Lake Superior water was also studied to determine if there might be any toxicological problems associated with using $NaHCO_3$ or Na_2CO_3 to increase alkalinity independently of hardness in later experiments.

The set of four tests consisted of 96 hour LC50 determinations and 7 day growth studies on fathead minnow larvae in four types of test water: ambient

Lake Superior water and separate 2 x 10^{-3} M concentrations of $CaCl_2$, $MgCl_2$ and NaCl in Lake Superior water. The chloride salts of Ca^{+2} and Mg^{+2} were added to the test waters instead of bicarbonate salts, so that increases in hardness could be obtained without the associated increases in alkalinity normally encountered in natural waters. Because the stability constants for copper chloride complexes are low, the addition of 4 x 10^{-3} M chloride should not have any significant effect on copper speciation.

The LC50 values in terms of total, dissolved, and free (Cu^{+2}) copper that were determined in lake waters with separate 2 x 10^{-3} M concentrations of $CaCl_2$, $MgCl_2$ and NaCl were all greater than those determined in ambient lake water, but the effects of $CaCl_2$ and of NaCl were substantially greater than the effect of $MgCl_2$ (Table 3-1). For example, the ratios of the dissolved copper LC50s in 2 x 10^{-3} M $CaCl_2$, $MgCl_2$ and NaCl to that in ambient lake water were, respectively, 3.9, 1.4, and 2.5 (Table 3-1). The ratios of Cu^{+2} LC50s in 2 x 10^{-3} M $CaCl_2$, $MgCl_2$, and NaCl to that in ambient lake water were, respectively, 9.5, 2.9, and 6.5. Figure 3-1 contains four plots of the negative logarithm of the Cu^{+2} activity versus the negative logarithm of the dissolved copper, one for each of the four test waters. The arrows on the Y and X axes show the relative positions of the negative logarithms of the LC50 values in terms of the Cu^{+2} activity and dissolved copper, respectively, for the four test waters.

The results of the $Ca+^2$ and Mg^{+2} tests (in which hardness in both waters was approximately 250 mg/L as $CaCO_3$ which is approximately five times greater than in ambient lake water) suggest that Ca^{+2} is much more responsible on an equivalent basis for the correlations between observed reductions in copper toxicity and hardness than Mg^{+2}. Therefore, separate measurements of Ca^{+2} and Mg^{+2} may be of greater value in predicting copper toxicity than hardness determinations, particularly if the Ca^{+2}/Mg^{+2} ratio changes substantially with time in the same natural water or with different natural waters.

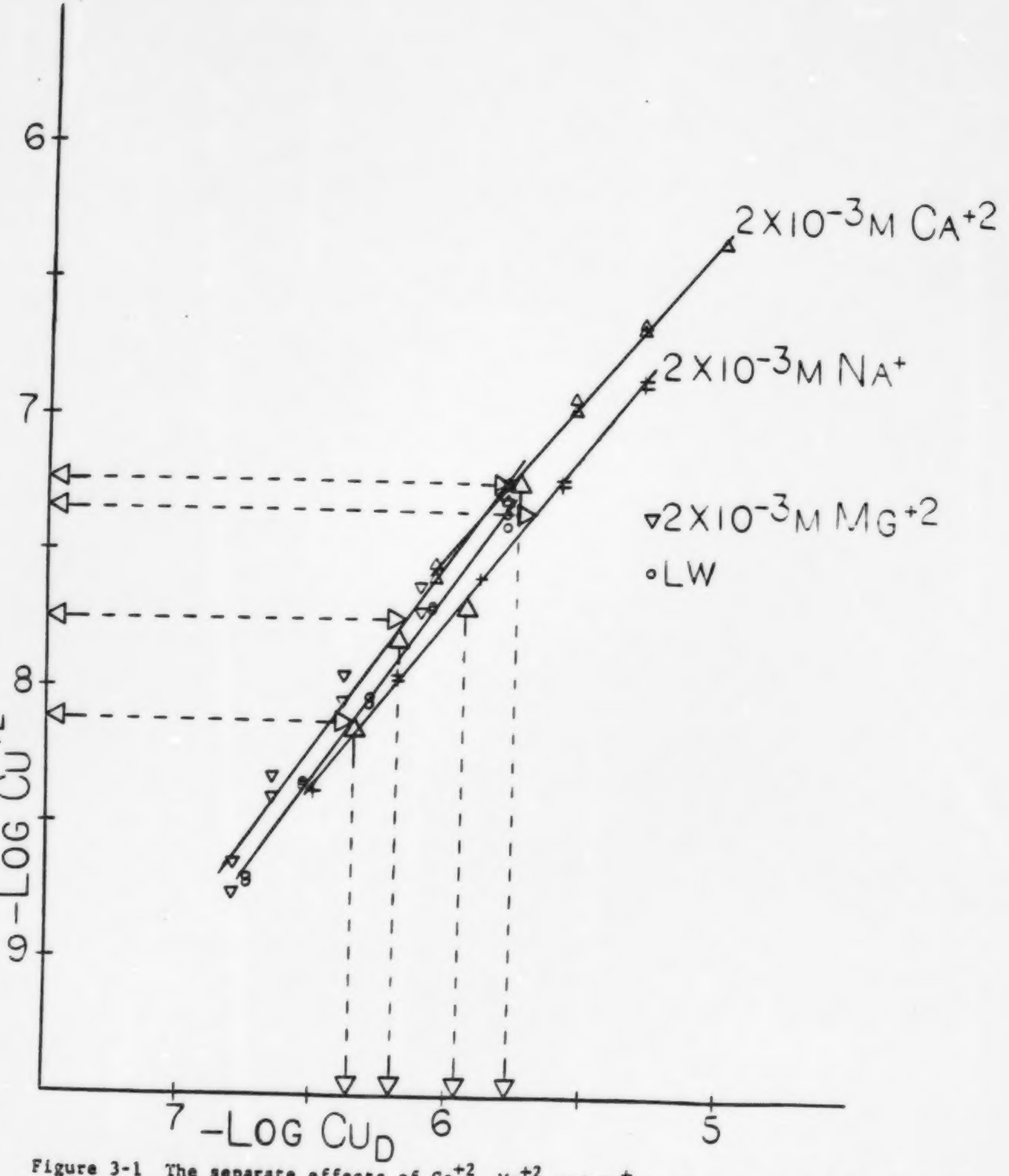

Figure 3-1 The separate effects of Ca^{+2}, Mg^{+2} and Na^{+} on the negative logarithms of Cu^{+2} and dissolved copper LC50s and in Lake Superior water

The large increase in the LC50 values in lake water with the addition of 2×10^{-3} M NaCl (which had no effect on hardness) indicated that Na^+ could reduce copper toxicity on an equivalent basis (2×10^{-3} N NaCl compared to 4×10^{-3} N $CaCl_2$) to an even greater extent than Ca^{+2}. Therefore, the Na^+ test indicated that neither $NaHCO_3$ nor Na_2CO_3 could be used for increasing the alkalinity in future tests, unless an additional chemical substituent which could nullify the effect of Na^+ on copper toxicity was added with the $NaHCO_3$ or Na_2CO_3. The 2×10^{-3} M concentration of Na^+ which was used in the experiment was much greater than would normally be found in any natural freshwater. Therefore, the effect of more typical concentrations of Na^+ on copper toxicity would probably be small compared to Ca^{+2} and Mg^{+2}, even in relatively soft waters.

In going from the ambient lake water to the 2×10^{-3} M $CaCl_2$, the calculated proportions (percentage/100) at the LC50 point of dissolved copper due to Cu^{+2} and the other major inorganic copper species increase. However, the calculated proportion of dissolved copper due to the theoretical copper-organic fraction decrease (Table 3-3) possibly due to competition between the Ca^{+2} and Cu^{+2} for organic ligands. Therefore, by referring to the first grouped term of equation (1-22), it can be seen that it is possible that the increase in the dissolved copper LC50 in going from ambient lake water to 2×10^{-3} M $CaCl_2$ lake water is at least partially due to the positive contributions of the negative proportional changes of possibly toxic organic compounds significantly offsetting the negative contributions of the positive proportional changes of any toxic inorganic copper species.

The increase in the dissolved copper LC50 in going from ambient lake water to 2×10^{-3} M $CaCl_2$ lake water appears to also be at least partially due to decreases in the toxicity/unit concentration of one or more toxic copper species as represented by the second grouped term of equation (1-22). The postulate is supported by the chemical speciation calculations listed in Table 3-2. The calculated concentrations at the LC50 point of Cu^{+2} and all of the other known major inorganic copper species are 5 to 10 times greater in the 2×10^{-3} M $CaCl_2$ lake water than in the ambient lake water. Also, the calculated concentration of the theoretical copper-organic fraction is greater in the 2×10^{-3} M $CaCl_2$ lake water than in the ambient lake water. Therefore, if the calculations are even just qualitatively correct in showing that all of the concentrations of major inorganic species and the concentration of the copper-organic fraction increase, the toxicity/unit concentration (e.g., T_i in equation 1-11) of one or more toxic copper species would have to decrease in order for equation (1-14) to be fulfilled. The reason is that if all of the toxicities/unit concentration remained constant in going from ambient lake water to the 2×10^{-3} M $CaCl_2$ lake water, all of the overall fractional contributions f_i of each species to toxicity would increase as can be seen from equation (1-15) because the concentration of each of the major inorganic species and the copper-organic fraction appear to increase. Therefore, if all of the fractional contributions increased, all of the f_i would be positive such that equation (1-14) could not be fulfilled.

Although the calculations of the concentrations of major inorganic copper species may have some significant quantitative error, the calculated increases (e.g., 5-10 times) are so large that it is unlikely that any of the concentrations of the major inorganic species decreased. Of course, the calculated increase in the concentration of the copper-organic fraction was not as large, and is probably subject to much larger error. Therefore, it is possible that the concentration of the copper-organic fraction was lower in the 2×10^{-3} M $CaCl_2$ lake water than in the ambient lake water, and that the copper-organic fraction is toxic. However, even if that is the case, if it is assumed that the toxicities/unit concentration remain constant, equation (1-17) would apply so that the product of the toxicity/unit concentration times the decrease in concentration (if any) of the copper-organic fraction would have to equal the sum of the product of the toxicity/unit concentration times the increase in concentration of each major inorganic copper species. Although possible, it is not probable. The reason is that the calculated increase in the total concentration of the inorganic copper fraction in going from ambient lake water to 2×10^{-3} M $CaCl_2$ is large ($+1.24 \times 10^{-6}$ M) compared to the calculated total concentrations of the copper-organic fraction in either water (0.21×10^{-6} M in ambient, 0.25×10^{-6} in 2×10^{-3} M $CaCl_2$). Therefore even if there is a relatively large error in the calculated concentrations of the copper-organic fraction and there is an actual decrease in the concentration of the copper-organic fraction in going from ambient lake water to the 2 x 10^{-3} M $CaCl_2$ instead of the calculated increase, it is not likely that the magnitude of such a decrease would be comparable to the increase in the total concentration of the inorganic copper fraction. Therefore, in order for equation (1-17) to hold, which is based on an assumption that all of the toxicities/unit concentration remain constant, the toxicity/unit concentration of the copper-organic fraction would have to be much greater than the concentration change weighted average of the toxicities/unit concentration of the inorganic copper species. That is unlikely particularly since the concentration of Cu^{+2}, which is postulated to be a very toxic species, increases substantially and therefore potentially contributes significantly to the concentration change weighted average of the toxicity/unit concentration of the inorganic fraction.

The discussions above support the postulate that the increase in the dissolved copper LC50 in going from ambient lake water to 2 x 10^{-3} M $CaCl_2$ lake water is due to a decrease in the proportion of a theoretical toxic copper-organic fraction and/or to a decrease in the toxicity/unit concentration of one or more toxic copper species. Although it is impossible from the present data set to determine the relative contributions of each possible cause to the increase in the LC50, it is certain that the increase in the LC50 cannot be contributed to by proportional changes of the known major inorganic species since they all increase. Furthermore, the contribution, if any, of any proportional decreases of copper-organic complexes appears to be small. Therefore, it appears probable that a decrease in the toxicity/unit concentration of one or more toxic copper species does occur which contributes significantly to the increase in the LC50.

If Ca^{+2} does significantly decrease the toxicity/unit concentration of one or more toxic copper species as was implied by the chemical speciation calculations, it would be necessary in developing the chemical speciation method of predicting LC50 values to determine several sets of toxicities/unit concentration, one set for each of several values of hardness normally encountered in natural waters. The advantage in that case of developing a more empirical toxicities factors method is clear since any changes in the toxicity/unit concentration of toxic copper species would affect, and therefore be reflected in, the observed functional dependence of experimental LC50 values on hardness. However, if substantial amounts of toxic copper-organic or other unknown toxic copper complexes are also present it would be extremely difficult, and perhaps impossible, to develop either a chemical speciation method or a more empirical toxicity factors method which could be used to accurately predict LC50 values in waters.

3.2 SET 2 - THE EFFECTS OF pH ON COPPER TOXICITY IN LAKE SUPERIOR WATER AT AMBIENT ALKALINITY

The objective of the second set of copper toxicity experiments was to determine the effect of pH on copper toxicity in Lake Superior water at ambient alkalinity and hardness. The four types of test waters used were: ambient Lake Superior water with an average pH of 8.06 and Lake Superior waters with the pH altered to an average pH of 6.58, 7.35, and 8.72, respectively. The pH was decreased from the ambient pH without decreasing alkalinity by bubbling CO_2 into the test waters. The amount of NaOH required to raise the pH to 8.72 from the ambient pH of 8.06 was not sufficient to cause any significant increase in alkalinity over that of the ambient Lake Superior water.

The LC50 values in terms of total and dissolved copper listed in Table 3-1 for set 2 test waters are similar and increase monotonically by a factor of more than 10 with increasing pH over the entire range of pH tested from 6.58 to 8.72. However, the LC50 values in terms of Cu^{+2} activity and concentration increase by a factor of approximately 2 from pH 6.58 to 7.35, level off between pH 7.35 and 8.06, and decrease by a factor of approximately 4 from pH 8.06 to 8.72. The somewhat unusual functional dependence of the Cu^{+2} LC50s on pH compared to the monotonic changes in the dissolved copper LC50 may be reflective of a gradual reduction in the contribution of toxicity/unit concentration changes to the monotonic increase in the dissolved copper LC50s compared to the contributions of proportional changes. Figure 3-2 contains four plots of the negative logarithm of the Cu^{+2} activity versus the negative logarithm of the dissolved copper, one for each of the four test waters in set 2. The arrows on the Y and X axes show the relative positions of the negative

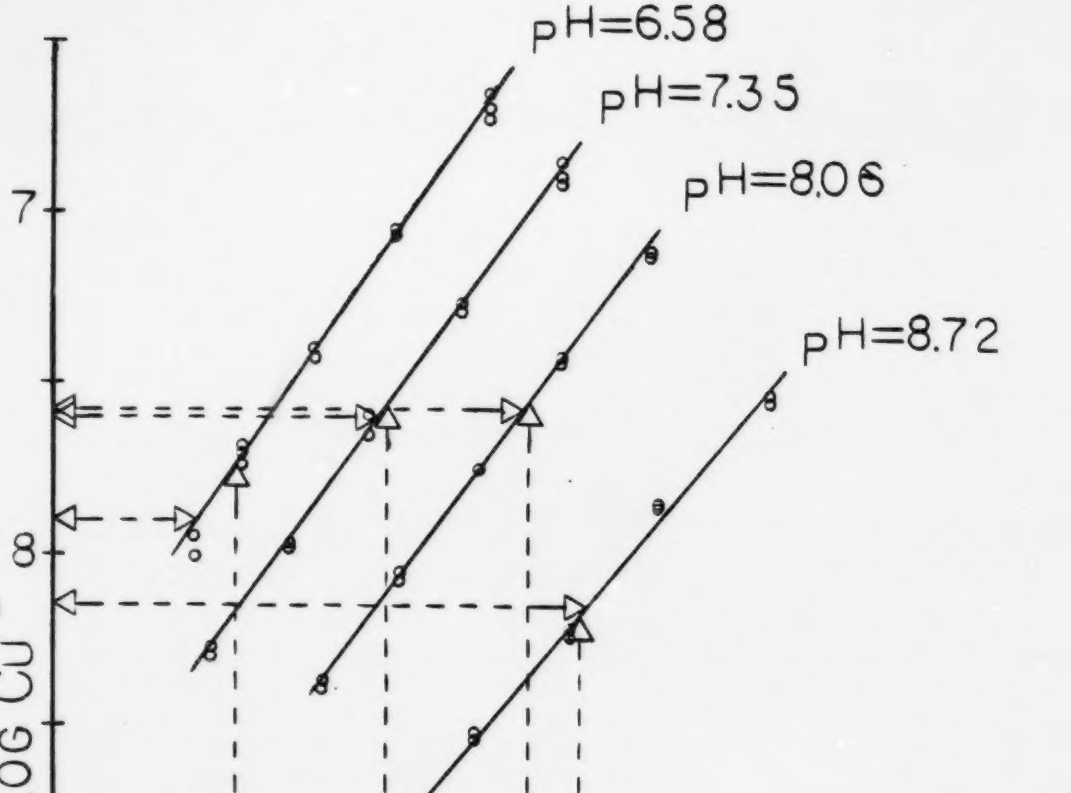

Figure 3-2 The effect of pH on the Negative logarithms of Cu^{+2} and dissolved copper LC50s in Lake Superior Water at 3X ambient alkalinity

logarithms of the LC50 values in terms of the Cu^{+2} activity and dissolved copper, respectively, for the four test waters.

As can be seen from the calculations for set 2 listed in Table 3-3, the proportions at the LC50 point of dissolved copper due to copper hydroxy and copper carbonate species increase with increasing pH over the range 6.58 to 8.06. However, the calculated proportions of dissolved copper due to Cu^{+2} decreases and that due to the theoretical copper-inorganic fraction greatly decreases possibly due to the increased competition between CO_3^{-2}, OH^-, and organic ligands for Cu^{+2}. The signs of the proportional changes between pH 8.06 and 8.72 are somewhat different in some cases, so they will be discussed separately. The observed proportional changes between pH 6.58 and 8.06 along with the first grouped term of equation 1-22 suggest that the increase in the dissolved copper LC50s over that pH range is at least partially due to the positive contributions of the negative proportional changes of Cu^{+2}. It may also be due to possibly toxic copper-organic complexes significantly off-setting the negative contributions of the positive proportional changes of copper hydroxy and copper carbonate species.

It is also probable that reductions in the toxicity/unit concentration of one or more toxic copper species, as represented by the second grouped term of equation 1-22, do occur and contribute significantly to the increase in the LC50 values. The reasoning is analogous to that for the set 1 experiments although the support for the postulate is not quite as great as that for the Ca^{+2} effect. The calculated concentrations listed in Table 3-2 for set 2 tests show the following. The calculated concentration at the LC50 point of Cu^{+2} increases from pH 6.58 to 7.35 by a factor of over 3 and then remains relatively constant from pH 7.35 to 8.06. The calculated concentration of the theoretical copper-organic fraction increases by a factor of less than 2 from pH 6.58 to 7.35, and then decreases by a factor of less than 2 from pH 7.35 to 8.06. The calculated concentrations at the LC50 point of all of the other known major inorganic copper species (e.g., copper hydroxy and copper carbonate species) increase by a factor of well over 10 to well over 100 from pH 6.58 to 8.06. If the concentrations of all the major inorganic copper species increase or remain constant and the concentration of the theoretical copper organic fraction increases or the copper-organic fraction is non-toxic from pH 6.58 to 8.06, there would have to be a decrease in the toxicity/unit concentration of one or more toxic species in order for equation (1-14) to be fulfilled. Of course, the calculated concentration of the copper organic fraction at the LC50 point decreases from pH 7.35 to 8.06, and the actual concentration of the organic fraction may decrease from pH 6.58 to 8.06 despite the increase in the calculated concentration for pH 6.58 to 7.35. That is because as discussed earlier, there may be large errors associated with the calculation of the concentration of the copper-organic fraction. However, even if that is the case, if it is assumed that the toxicities/unit remain constant, equation (1-17) would apply so that the product of the toxicity/unit concentration times the decrease in concentration (if any) of the copper-organic fraction would have to equal the sum of products of the toxicity/unit concentration times the increase in concentration for each major inorganic copper species. Again, as was the case for Ca^{+2} addition, the increase in the calculated total concentration of the inorganic copper fraction from pH 6.58 to 8.06 ($+6.71 \times 10^{-7}$ M) is large compared to the calculated concentrations of the theoretical copper-organic fraction at pHs 6.58, 7.35 and 8.06 (0.85×10^{-7}, 1.41×10^{-7}, 0.91×10^{-7} M, respectively). Therefore, even if there is a relatively large error in the calculated concentrations of the copper-organic fraction and the concentration of the copper-organic fraction decreases with increasing pH from 6.58 to 8.06, it is unlikely that the magnitude of any such decrease would be comparable to the increase in the concentration of the total inorganic copper fraction. Therefore again, in order for equation (1-17) to hold which is based on the assumption that all of the toxicities/unit concentration remain constant, the toxicity/unit concentration (or more accurately the concentration change weighted average toxicity/unit concentration) of the copper-organic fraction would have to be much greater than the concentration change weighted average toxicity/unit concentration of the copper inorganic fraction. That is again, unlikely, particularly from pH 6.58 to 7.35 where the apparently toxic Cu^{+2} concentration significantly increases and therefore potentially contributes significantly to the concentration change weighted average toxicity/unit concentration of the inorganic copper fraction. The argument is not as strong for pH 7.35 to 8.06 since the calculated concentration of Cu^{+2} at the LC50 point remains almost constant and therefore contributes little (if any) to the concentration change weighted average toxicity/unit concentration of the inorganic copper fraction.

The discussions above concerning the pH range from 6.58 to 8.06 indicate that increases in the dissolved copper LC50 over that range are due to negative proportional changes of Cu^{+2} and possibly toxic copper-organic complexes, and/or to decreases in the toxicity/unit concentration of one or more toxic copper species. Again, as with the Ca^{+2} effect, it is not possible from the present set of data to determine the relative contributions of the two possible causes to the increase of dissolved copper LC50s with increasing pH. Unlike for the Ca^{+2} effect, the proportion of an inorganic species (Cu^{+2}) decreases with increasing pH and therefore could contribute to the increase in the dissolved copper LC50s. Furthermore, the species involved (Cu^{+2}) has been postulated to be toxic and its proportion decreases substantially. Therefore, the proportional decrease in Cu^{+2} from pH 6.58 to 8.06 could contribute significantly to the increase in the dissolved copper LC50s. However, the proportional decrease in Cu^{+2} cannot completely account for the observed increase. The reason is that despite the negative proportional change of Cu^{+2}, the concentration of Cu^{+2} increases from pH 6.58 to 7.35 and remains almost constant from pH 7.35 to 8.06, whereas the concentrations of all of the other known major inorganic complexes greatly increase. Therefore, it would be impossible for equation (1-14) to be fulfilled, unless there was a significant decrease in the concentration of toxic copper-organic complexes and/or in the toxicities/unit concentration of one or more toxic copper species. Furthermore, because any decrease in copper organic complex concentrations appears to be small compared to the increases in the concentrations of the inorganic complexes, it appears likely that decreases in the toxicity/unit concentration of one or more toxic copper species do occur in going from pH 6.58 to 8.06, and do contribute significantly to the increase in the dissolved copper LC50.

Although it was shown that it is not possible for the decrease in the proportion of Cu^{+2} from pH 6.58 to 8.06 to completely account for the increase in dissolved copper LC50s, it is conceivable that the negative proportional decrease in Cu^{+2} could account for all, or at least most, of the observed increase in the dissolved copper LC50s from pH 8.06 to 8.65. The reason is that in this case, the concentration of Cu^{+2} does significantly decrease along with the negative proportional change. Therefore, equation (1-14) could conceivably be fulfilled by the decrease in Cu^{+2} concentration alone without having to assume that the toxicities/unit concentration of one or more species decreases, and/or that the concentrations of some toxic copper-organic complexes (if any) decrease. If that was true, it can be shown from equation (1-17) which would then apply to the pH range, that the toxicity/unit concentration of Cu^{+2} would have to be >14 times the concentration change weighted average toxicity/unit concentration of the remaining dissolved copper. Furthermore, since the increase in concentration of $Cu(OH)_2^{\circ}$, $CuCO_3^{\circ}$ and $Cu(CO_3)_2^{-2}$ are all much greater than the decrease in the Cu^{+2} concentration, the toxicity/unit concentration of Cu^{+2} would have to be much greater than for any of those species.

If increasing pH does decrease the toxicity/unit concentration of one or more toxic copper species, it would probably not be possible to develop a chemical speciation method of estimating LC50s. The reason is that in order to calculate the toxicities/unit concentration of copper hydroxy species, it is necessary to vary the relative proportion of those species compared to Cu^{+2} which can only be done through varying the pH. However, if by varying the pH, the toxicities/unit concentration also change, it would not be possible to determine them. Therefore, unless the toxicities/unit concentration of all of the major copper hydroxy species are negligible, a chemical speciation method could not be developed. However, any such effects of pH on the toxicities/unit concentration should not interfere in the development of a toxicity factors method, since they would affect and therefore be reflected by the dependence of LC50 values on pH. If, however, there are significant amounts of toxic copper-organic or other unknown toxic complexes present, it would be extremely difficult to develop either a chemical speciation or an accurate toxicity factors method.

3.3 SET 3 - THE EFFECTS OF pH ON COPPER TOXICITY IN LAKE SUPERIOR WATER WITH THE ALKALINITY INCREASED TO APPROXIMATELY THREE TIMES THE AMBIENT ALKALINITY

There were three major objectives of the third set of copper toxicity tests. The first objective was to determine the effect of pH on copper toxicity in waters with an intermediate alkalinity. The second objective was to determine the effect of alkalinity on copper toxicity at a constant, lower than ambient lake water pH. The third objective was to determine the effect of alkalinity on the pH dependence of copper toxicity by comparing the results of the tests run in the third set at intermediate alkalinity to those of the tests run in the second set at the lower ambient lake water alkalinity.

The first three test waters in the third set of copper toxicity tests were all Lake Superior waters with alkalinities increased to approximately three times (e.g., 3×10^{-3} eg,/L) that of ambient lake water and pHs adjusted to an average pH of 7.15, 7.94 and 8.56, respectively. The pH 7.94 is similar to that of ambient lake water. The alkalinities of the first three test waters were increased without increasing the ambient hardness of lake water by adding a ratio of $NaHCO_3$ to $KHCO_3$ which did not appear to have any effect on copper toxicity based on preliminary static experiments with various ratios of Na^+/K^+ added. The K^+ apparently increases copper toxicity alone and therefore can offset the decrease in copper toxicity by Na^+ alone. The 8.56 pH was the unaltered steady state pH of the water when the alkalinity was increased to three times that of ambient lake water. The pHs of 7.94 and 7.15 were obtained without lowering the alkalinity by bubbling CO_2 into the test waters. In addition to the three test waters run in the third set at three times the ambient alkalinity, a fourth test water was run at approximately one-half (e.g., -0.5×10^{-3} eq/L) the alkalinity of ambient lake water and at a lower pH (7.15) than ambient lake water.

The effect of increasing pH on the LC50 values in the first three test waters in set 3 which were at intermediate alkalinity can be seen from Table 3-1. The LC50 values in terms of total and dissolved copper were similar at any given pH, and increased monotonically by a factor of over 7-fold with increasing pH over the entire range tested from pH 7.15 to 8.56. However, the

LC50 values in terms of the Cu^{+2} activity and concentration decreased monotonically by a factor of approximately 3-fold with increasing pH over the same range. Figure 3-3A contains four plots of the negative logarithm of the Cu^{+2} activity versus the negative logarithm of the dissolved copper, one for each of the four test waters in set 3. The arrows on the Y and X axes show the relative positions of the negative logarithms of the LC50 values in terms of the Cu^{+2} activity and dissolved copper, respectively, for the four test waters.

Plots of $-\log LC50_{DISS\ Cu}$ and $-\log LC50_{(Cu^{+2})}$ versus pH for both set 2 and set 3 test waters are presented in Figure 3-3B. The plots of $-\log LC50_{DISS\ Cu}$ vs pH for sets 2 and 3 are qualitatively similar in that both show monotonic increases with increasing pH and a reduction in the slope at higher pHs. The reduction in the slopes at higher pH may be due to a reduction in factors other than inorganic proportional changes contributing to the increase in the LC50s such as possibly a reduction in the contribution of toxicities/unit concentration changes. It could also be due to increases in the proportion of $Cu(OH)_2^{\circ}$ and/or $Cu(CO_3)_2^{-2}$ copper complexes as compared to $CuOH^{+}$ and/or $CuCO_3^{\circ}$ complexes if either or both of the di complexes has a greater toxicity/unit concentration than the corresponding mono complex.

The slope of the $-\log LC50_{DISS\ Cu}$ vs pH plot for set 3 waters run at approximately 3×10^{-3} eq/L alkalinity is steeper at all pHs tested than the slope of the plot for set 2 waters run at 1×10^{-3} eq/L alkalinity. Therefore, the two curves diverge and there is a gradual increase in the ratio of the dissolved copper LC50 at 3×10^{-3} eq/L alkalinity to the dissolved copper LC50 at 1×10^{-3} eq/L alkalinity with increasing pH ranging from approximately 1.25 at pH 7.15 to approximately 2.0 at pH 8.56. That is not surprising since much of the available literature suggests that the toxicity/unit concentration of Cu^{+2} and of $CuOH^{+}$ are far greater than that of $CuCO_3^{\circ}$ or $Cu(CO_3)_2^{-2}$. However, it is somewhat surprising that the apparent effect of alkalinity on the pH dependence of dissolved copper LC50s is not even greater than was observed. Furthermore, it appears that the observed effect may have been greater than the actual effect of alkalinity due to a possible decrease in the sensitivity of the organisms to copper in set 3 compared to set 2. Although a dissolved

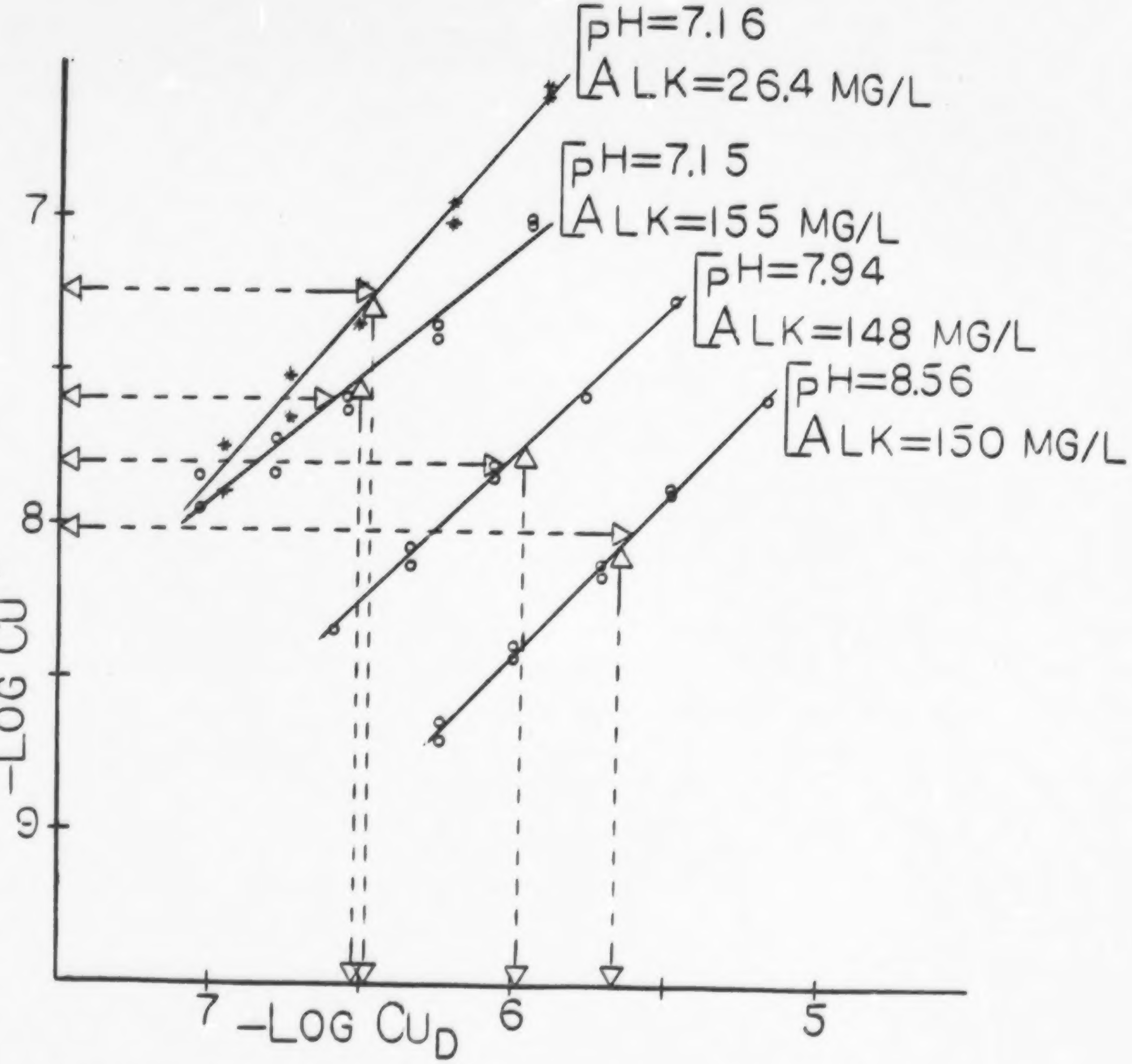

Figure 3-3A The effect of pH on the negative logarithms of Cu^{+2} and dissolved copper LC50s in the Lake Superior water at 3X ambient alkalinity

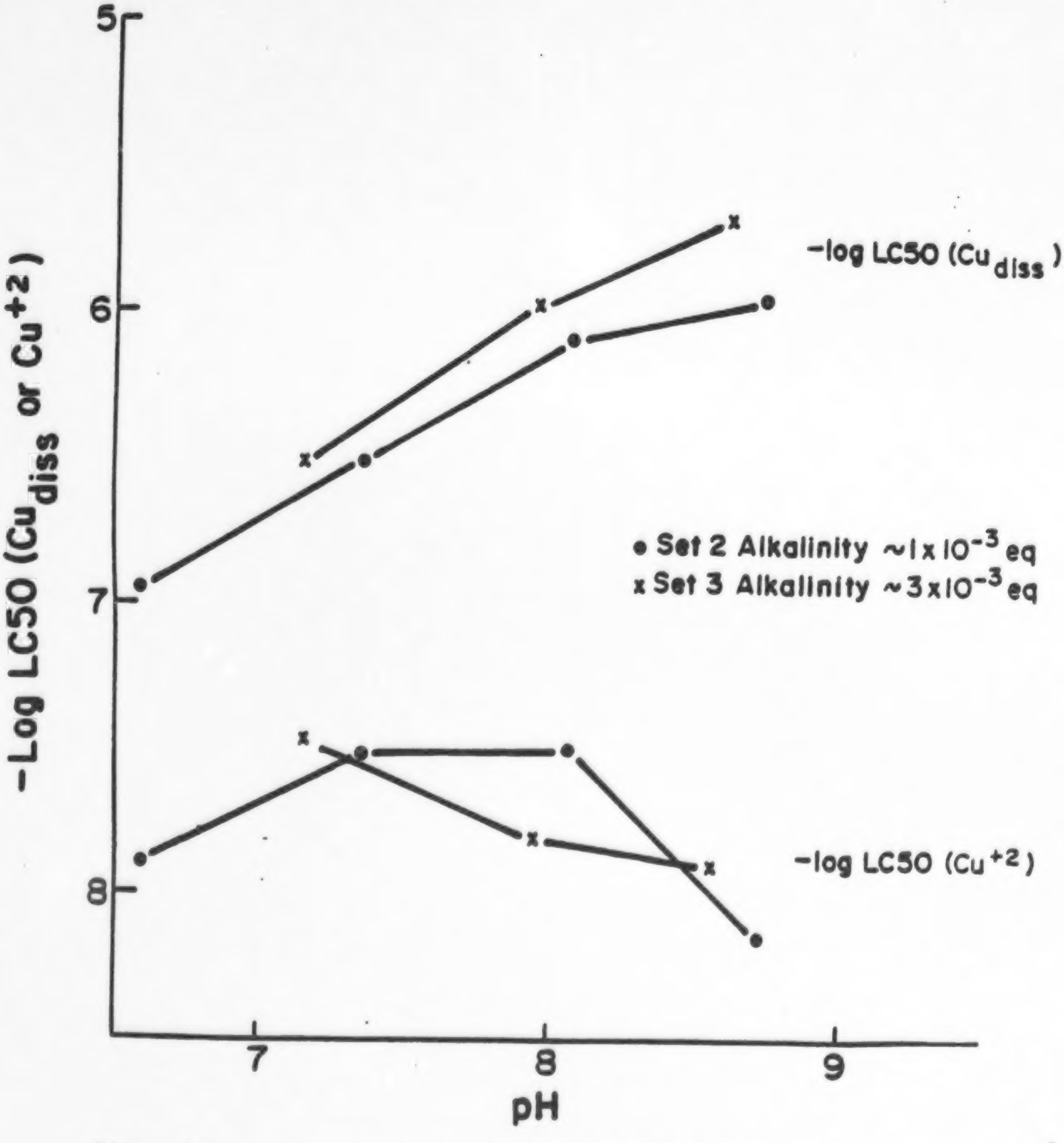

Figure 3-3B. A comparison of the effect of pH on the negative logarithms Cu^{+2} and dissolved copper LC50s in Lake Superior water at ambient and 3X ambient alkalinity

copper LC50 was not determined in ambient lake water in set 3, dissolved copper LC50s in ambient lake water steadily increase from set 1 to set 2 to set 4 which suggests that the organisms sensitivity to copper may have decreased in going from set 2 to set 3. If so, part of the increase in dissolved copper LC50s in going from set 2 at 1×10^{-3} eq/L alkalinity to set 3 at 3×10^{-3} eq/L at a given pH may have been due to a decrease in sensitivity causing a decrease in the toxicities/unit concentration of toxic species.

One of the reasons why the effect of alkalinity is not as great as expected may be due to substantial organic complexation in the lower alkalinity waters of set 2. For example, the dissolved copper LC50s of the second test water of set 2 and the first test water of set 3 are the same. Although the concentrations of $CuCO_3^{\circ}$ and $Cu(CO_3)_2^{-2}$ are much greater in the set 3 test water than in the set 2 test water, the concentrations of Cu^{+2} and of $CuOH^{+}$ are similar in the two waters. The reason appears to be that a somewhat comparable amount of copper is bound to organic ligands in the lower alkalinity water (Table 3-3) as was in the form of $CuCO_3^{\circ}$ and $Cu(CO_3)_2^{-2}$ in the higher alkalinity water. That, along with the identical dissolved copper LC50s and the comparable Cu^{+2} and $CuOH^{+}$ concentrations, suggest that the toxicity/unit concentration of the copper-organic fraction may be somewhat comparable to that of $CuCO_3^{\circ}$. Therefore, organic complexation in the lower alkalinity waters could have a similar effect on copper toxicity as $CuCO_3^{\circ}$ formation in the higher alkalinity and therefore could mask to some extent the effect of alkalinity.

The monotonic decrease in the Cu^{+2} LC50s with increasing pH as opposed to the non-monotonic dependence of Cu^{+2} LC50s on pH in set 2 as shown in Figure 3-3B, may be reflective of a greater contribution of decreases in the Cu^{+2} proportion to the increases in the dissolved copper LC50s in set 3 than in set 2. In set 2, other factors such as decreases in the proportion of toxic copper-organic complexes and/or decreases in toxicities/unit concentration may have contributed substantially to the increases in the dissolved copper LC50s. In particular, the near Nernstian slopes of the electrode response and the small or negative differences between the dissolved copper and the sum of calculated concentrations of known inorganic copper species (both of which are shown in Table 3-3) indicate that any copper-organic fraction present is probably very small. Therefore, it is unlikely that decreases in the proportion of toxic copper-organic complexes (if present) would contribute significantly to the observed increases in the dissolved copper LC50s. Of course, there might be decreases in the toxicity/unit concentration of toxic copper species which contribute significantly to the dissolved copper LC50 increase. However, there is not as much evidence to support that as there was in set 2, because equation (1-14) can conceivably be fulfilled by decreases in the Cu^{+2} concentration alone over the entire pH range tested.

If the toxicities/unit concentration are assumed to remain constant with increasing pH from pH 7.15 to 8.56, equation (1-17) will apply. If in addition the copper-organic fraction is assumed to be negligible, it can be shown that in order for equation (1-17) to hold over the pH range of 7.15 to 8.56, the product of the Cu^{+2} toxicity/unit concentration times the decrease in Cu^{+2} concentration would have to equal the sum of the products of the toxicity/unit concentration times the increase in concentration for each of the other inorganic copper species. By substituting values from Table 3-2 into equation (1-17), it can be shown that the toxicity/unit concentration of the Cu^{+2} would have to be greater than 88 times the concentration change weighted average toxicity/unit concentration of the rest of the dissolved copper if the toxicities/unit concentration remained constant. Furthermore, because the increases in the concentration of $CuOH^{+}$, $Cu(OH)_2^{\circ}$ and $Cu(CO_3)_2^{-2}$ are larger and the increase in the concentration of $CuCO_3^{\circ}$ is much larger than the decrease in the Cu^{+2} concentration, the toxicity/unit concentration of the Cu^{+2} would have to be greater than those for $CuOH^{+}$, $Cu(OH)_2$ and $Cu(CO_3)_2^{-2}$ and much greater than for $CuCO_3^{\circ}$.

Since we want to determine the effects of alkalinity on copper toxicity, it is interesting to note that even in the minimum case with the toxicities/unit concentration of the copper hydroxy species assumed to be zero, the toxicity/unit concentration of Cu^{+2} is still calculated to be at least 82 times greater than the concentration change average weighted toxicity/unit concentration of $CuCO_3^{\circ}$ and $Cu(CO_3^{\circ})_2^{-2}$. That does not mean of course that the fractional contribution of $CuCO_3^\circ$ and/or $Cu(CO_3)_2^{-2}$ to toxicity are necessarily negligible since the fractional contribution is equivalent to the toxicity/unit concentration times the concentration. Therefore if the ratio of the sum of $CuCO_3^\circ$ and $Cu(CO_3)_2^{-2}$ concentrations to the Cu^{+2} concentration is large such as in alkaline waters, they may still contribute significantly to toxicity.

The results from the fourth test water of set 3 run at one-half ambient alkalinity and a pH of 7.16 compared to the results from the first test water of set 3 run at three times ambient alkalinity and an almost identical pH of 7.15, indicate the following. In going from test water 1 to test water 4, the Cu^{+2} LC50 approximately doubles, the concentrations of $CuOH^+$, $Cu(OH)_2$ and Cu-Org increase and the concentrations of $CuCO_3^\circ$ and $Cu(CO_3)_2^{-2}$ decrease. If the concentration changes (Table 3-3) in going from test water 1 to test water 4 are substituted into equation (1-17) assuming that the toxicities/unit concentration remain constant, the following equation results

$$(3.4 \times 10^{-8})\ T_{Cu^{+2}} + (1.2 \times 10^{-8})\ T_{CuOH^{+}} + (2.7 \times 10^{-1}0)\ T_{Cu(OH)_2^{\circ}}$$
$$+ (1.4 \times 10^{-7})\ T_{CuOrg} = (1.5 \times 10^{-7})\ T_{CuCO_3^{\circ}} + 1.7 \times 10^{-9})\ T_{Cu(CO_3)_2^{-2}}$$

Even in the maximum case with the toxicities/unit concentration of $CuOH^+$, $Cu(OH)_2^\circ$ and Cu-Org assumed to be zero, the toxicity/unit concentration of Cu^{+2} is calculated to be only approximately 4.5 times the concentration change average toxicity/unit concentration of $CuCO_3^\circ$ and $Cu(CO_3)_2^\circ$ compared to the earlier estimated ratio of greater than 82.

One possible reason for the discrepancy is that the organisms in test water 1 may have been under stress from the relatively high estimated concentration of CO_2 (21 mg/L) in the water due to the combination of relatively high alkalinity and low pH. If that was the case, the toxicities/unit concentration in going from test water 1 to test water 4 may have decreased so that equation (1-17) would no longer apply. Also, it is possible that toxicities/unit concentration also decreased with increasing pH in going from test water 1 to test water 3.

3.4 SET 4 - THE SEPARATE AND JOINT EFFECTS OF HUMICS AND SUSPENDED CLAY ON COPPER TOXICITY IN LAKE SUPERIOR WATER

The objective of the fourth set of copper toxicity tests was to determine the separate and joint effects of humics and suspended clays on copper toxicity in Lake Superior water. The four test waters used in set 4 were: ambient Lake Superior water with negligible amounts of humics and suspended clay, ambient lake water with Lake Superior shore red clays added in suspension until a turbidity of 70 NTU was obtained, ambient lake water with humics purchased from Aldrich added to a nominal TOC of 5 mg/L, and ambient lake water with both the clay and humics added at the previous separate levels. The addition of the clay suspension increased the average pH of the ambient lake water between 0.15 and 0.20 pH units possibly due to the exchange of H^+ with cations from the clay, but did not have any significant effect on any other general water quality parameters. The addition of the humics did not have any significant effect on the pH or any other general water quality parameter.

Table (3-1) shows that in going from ambient lake water to the suspended clay water, the total copper LC50 increases approximately 50%, whereas the dissolved copper LC50 decreases approximately 25% and the Cu^{+2} LC50 decreases by over 50%. Figure 3-4 contains four plots of the negative logarithm of the Cu^{+2} activity versus the negative logarithm of the dissolved copper, one for each of the four test waters in set 4. The arrows on the y and x axes show the relative positions of the negative logarithms of the LC50 values in terms of the Cu^{+2} activity and dissolved copper, respectively, for the four test waters.

Since the suspended clay can adsorb copper to some extent, the increase in the total copper LC50 is not surprising. However, since the clay suspension increased the pH of the ambient lake water, the decrease in the dissolved copper LC50 was somewhat unexpected because the dissolved copper LC50s increased with increasing pH in set 2 and set 3 tests. Furthermore, in looking at the chemical speciation calculations in Table (3-2), it can be seen that the concentrations of Cu^{+2} at the LC50 point and all of the other known major inorganic species except $Cu(CO_3)_2^{-2}$ and $Cu(OH)_2^{\circ}$ decrease substantially in going from the ambient lake water to the suspended clay lake water, and

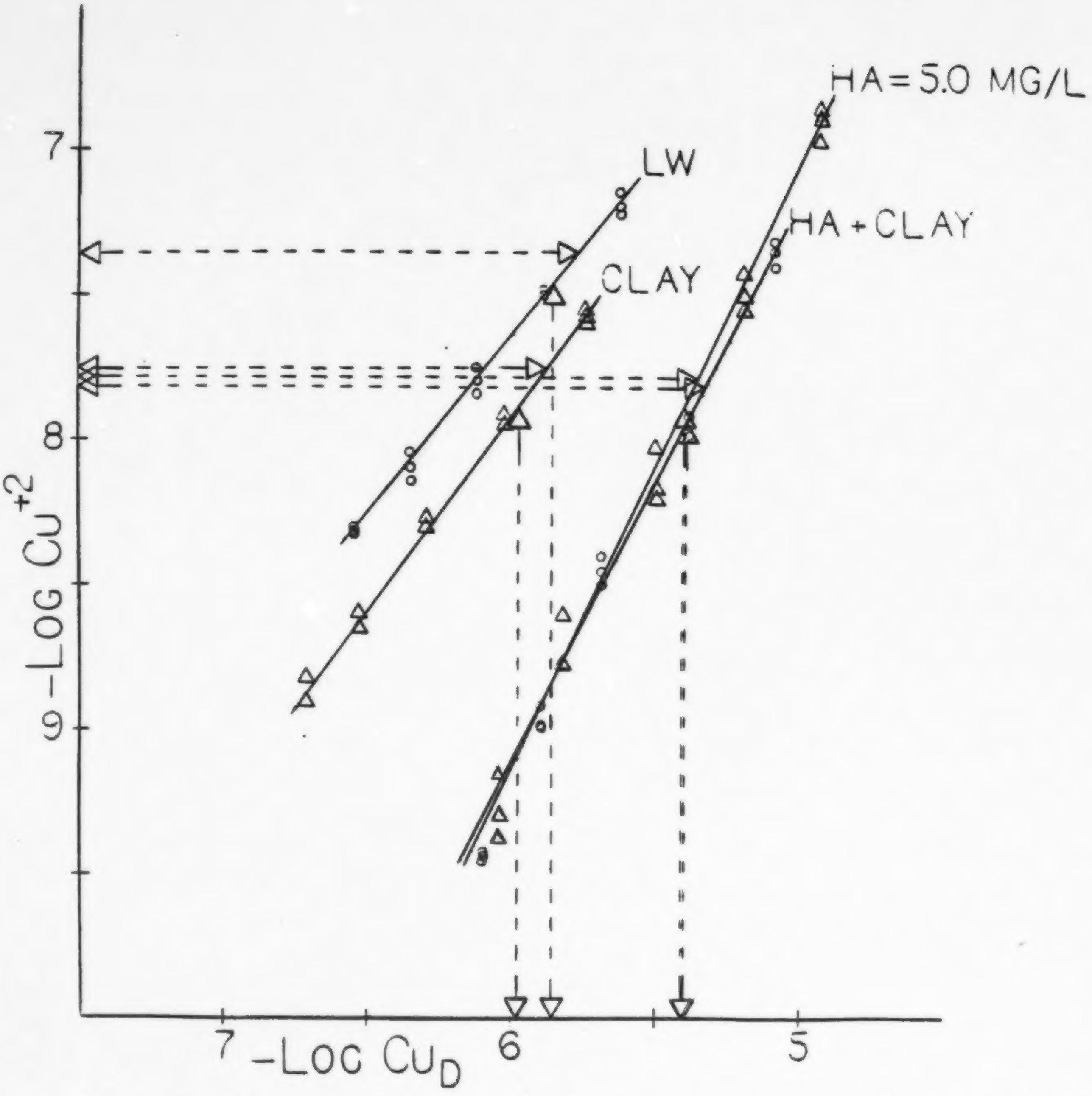

Figure 3-4 The separate and joint effects of humics and suspended clay on the negative togarithm of Cu^{+2} and dissolved copper LC50s

that the calculated concentration of the theoretical copper-organic fraction also decreases slightly. The calculated concentrations of $Cu(OH)_2$ stays approximately the same and the calculated concentration of $Cu(CO_3)_2^{-2}$ increases, but to a small extent compared to the decreases in concentrations of Cu^{+2}, $CuOH^+$ and $CuCO_3°$. Therefore, if the toxicities/unit concentration are assumed to remain constant and if any copper-clay complexes formed are assumed to be non-toxic, the toxicity/unit concentration of $Cu(CO_3)_2^{-2}$ would have to be many times greater than the concentration change weighted average toxicity/unit concentration of the rest of the dissolved copper for equations (1-14) and (1-17) to hold. Furthermore, since the decrease in Cu^{+2} concentration is approximately 10 times the increase in $Cu(CO_3)_2^{-2}$, the toxicity/unit concentration of $Cu(CO_3)_2^{-2}$ would have to be over 10 times greater than for Cu^{+2} for equation (1-17) to hold. That is unlikely considering that the results of previous tests have suggested that the toxicity/unit concentration of $Cu(CO_3)_2^{-2}$ is lower than for Cu^{+2}.

The above discussion suggests that in order for equation (1-14) to hold in going from ambient lake water to suspended clay lake water, either the toxicity/unit concentration of one or more toxic copper species must increase, or some of the suspended copper-clay complexes must be toxic, or both.

Table (3-1) shows that in going from the ambient lake water to the humics lake water, both the total and dissolved copper LC50s increase by a factor of approximately three fold, whereas the Cu^{+2} LC50 decreases by over 60%. Humics have a relatively large binding affinity for copper and do not appear to affect general water quality parameters. Therefore, the rather large increases in the total and dissolved copper LC50s in going from ambient lake water to the humics lake water is not surprising. However, again as with the clay, the concentrations of Cu^{+2}, $CuOH^+$ and $CuCO_3°$ decrease substantially and in this case, the concentrations of $Cu(OH)_2°$ and $Cu(CO_3)_2°$ decrease as well. Therefore, in order for equation (1-14) to be fulfilled in going from ambient lake water to humic lake water, either the toxicity/unit concentration of one or more toxic copper species must increase or at least some of the copper-humic complexes must significantly contribute to toxicity, or both.

Although the humics addition does not significantly effect the hardness determination possibly because of the EDTA titration stripping any humic bound Ca^{+2} or Mg^{+2}, the humics may substantially reduce the concentration of Ca^{+2} and/or Mg^{+2} which could increase the toxicities/unit concentrations of the toxic copper species (83). Also copper-humic complexes could possibly contribute substantially to toxicity but again through, some external mechanism since dialysis experiments (82) have shown that virtually all of the cadmium-humic complexes have molecular weights exceeding 1000 and are, therefore, unlikely to be transported across gill membranes.

Table (3-1) shows that in going from ambient lake water to the humics plus suspended clay water, the increase in the total copper LC50 is almost equal to the sum of the separate increases in going to the suspended clay water and in going to the humics water. The dissolved copper LC50 increases by a factor of approximately three, whereas the Cu^{+2} LC50 decreases by approximately 60%. The concentrations of all of the major inorganic forms of copper decrease except for $Cu(CO_3)_2^{-2}$ which increases slightly. Therefore, by an argument analogous to that given above, it is unlikely that equation (1-14) could be fulfilled without the toxicity/ unit concentration of one or more toxic copper species increasing and/or copper-humic complexes contributing significantly to toxicity.

In going from the humics water to the humics plus suspended clay water, the dissolved copper LC50 and the Cu^{+2} LC50 remained almost constant despite the increases in the concentrations of all of the other inorganic copper species. This suggests that while the combined effect of humics and suspended clays on copper toxicity in terms of total copper is almost additive, the combined effects in terms of dissolved copper and Cu^{+2} are at best, no more than the effects of humics alone. That is somewhat surprising since it appears that the sole addition of suspended clay to lake water does lead to an increase in the toxicity/unit concentration of toxic copper species and/or to the formation of toxic copper-clay complexes as discussed earlier. However, the presence of substantial amounts of humics appears to almost completely nullify such suspended clay effects and reduce the effects to that of adsorption alone.

3.5 SET 5 - THE EFFECTS OF VARIABLE HUMIC CONCENTRATIONS ON COPPER TOXICITY IN LAKE SUPERIOR WATER

The objective of set 5 was to determine the effect on copper toxicity of humic concentrations that were lower than the 5.0 mg/L used in set 4, because the reduction in the Cu^{+2}LC50 observed in set 4 for that humic concentration may have possibly been due to the humics stresssing the organism. The four test waters of set 5 consisted of ambient lake water and lake water to which humics were added to a nominal TOC of 1.25 mg/L, 2.50 mg/L and 5.0 mg/L.

Table (3-1) shows that both total and dissolved copper LC50s increase monotonically by over a factor of 3 with increasing humic concentration to 5 mg/L nominal TOC with the dissolved copper LC50 averaging approximately 80% of the total copper LC50. The Cu^{+2} LC50 decreases approximately 33% in going from ambient lake water to the 1.25 mg/L nominal TOC water, remains constant in going from the 1.25 mg/L nominal TOC water to the 2.50 mg/L nominal TOC water, and then decreases by approximately 50% in going from the 2.50 mg/L nominal TOC water to the 5.0 mg/L nominal TOC water. In going from the ambient lake water to any of the humic waters, the concentration at the LC50 point of Cu^{+2}, and all of the other major inorganic species decrease. Therefore, in order for equation (1-14) to be fulfilled, either the toxicities/unit concentration of one or more toxic copper species must increase, or at least some of the copper-humic complexes must contribute significantly to toxicity, or both. Figure 3-5A contains four plots of the negative logarithm of the Cu^{+2} activity versus the negative logarithm of the dissolved copper for the four test waters of set 5. The arrows of the Y and X axes show the relative positions of the negative logarithms of the LC50 values in terms of the Cu^{+2} activity and dissolved copper, respectively.

There is some evidence to support the postulate that the effects of the lower humic concentrations on copper toxicity may also be primarily through increases in toxicities/unit concentration rather than through contributions of copper-humic complexes to toxicity. The reason is that despite an over 100% increase in the large copper-organic fraction in going from the 1.25 mg/L nominal TOC water to the 2.50 mg/L TOC water, the concentrations at the LC50 point of Cu^{+2} and all of the other known major inorganic copper species remain

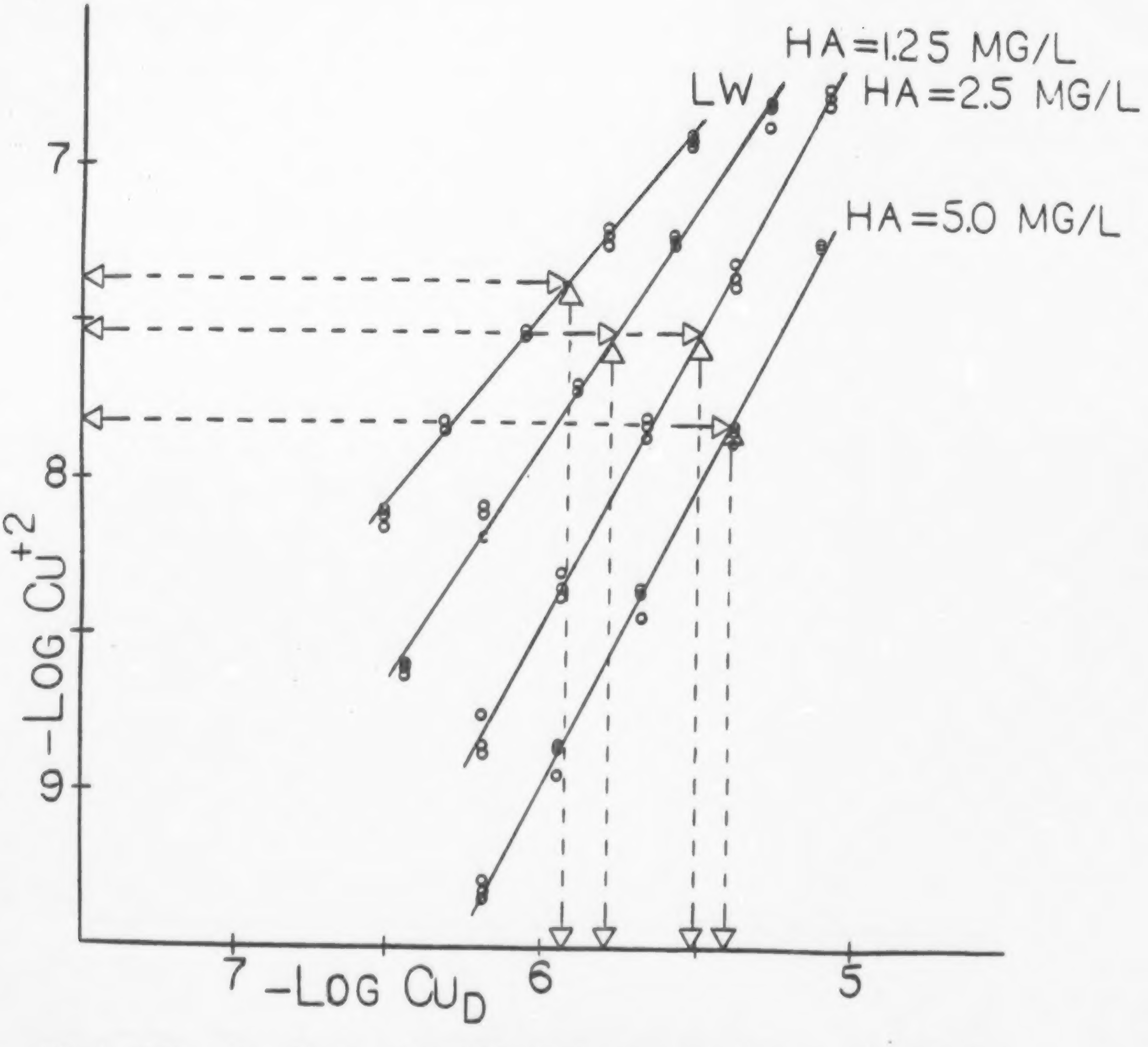

Figure 3-5A. The effects of variable humic concentrations on the negative logarithms of Cu^{+2} and dissolved copper LC50s in Lake Superior water

relatively constant, as can be seen in Table 3-2. That suggests that the copper-organic fraction does not contribute significantly to toxicity. It also suggests that the toxicities/unit concentration remain relatively constant in going from the 1.25 mg/L nominal TOC water to the 2.50 mg/L nominal TOC water even though they appear to possibly increase in going from the ambient lake water to either the 1.25 mg/L nominal TOC water or the 2.50 mg/L nominal TOC water.

The results of the set 5 copper toxicity tests suggest that humics may increase the toxicity/unit concentration of one or more toxic copper species. As mentioned previously, humics could possibly increase toxicities/unit concentration through the binding of Ca^{+2} and/or Mg^{+2} which appear to possibly decrease toxicities/unit concentration. However, if that is the case, the results of set 5 suggest that the 1.25 mg/L nominal TOC is capable of binding most of the Ca^{+2} and Mg^{+2} present because there does not appear to be any increase in the toxicities/unit concentration in going from the 1.25 mg/L nominal TOC water to the 2.50 mg/L nominal TOC water.

The effects of humics added to a nominal TOC of 2.5 mg/L, 10 mg/L, and 20 mg/L on cadmium toxicity in Lake Superior water are depicted in figure 3-5B. The figure contains four plots of the negative logarithm of the Cd^{+2} activity versus the negative logarithm of dissolved cadmium, one for Lake Superior water and three for the three waters with added humics. The arrows on the Y and X axes show the relative positions of LC50 values in terms of the Cd^{+2} activity and dissolved cadmium, respectively, for the four test waters. The figure shows that whereas increased in humic concentration increase dissolved cadmium LC50s, they decrease Cd^{+2} LC50s. Therefore, the effects of increasing humic concentration on cadmium toxicity are qualitatively identical to those on copper toxicity which are depicted in figure 3-5A.

By analogy to the earlier discussion on the effects of humics on copper toxicity, the results of the cadmium toxicity tests suggest that humics increase the toxicity/unit concentration of toxic cadmium species and/or that at least some cadmium-humic complexes are toxic. There were no apparent signs of stress on the organisms except in the 20 mg/L humics water where a statis-

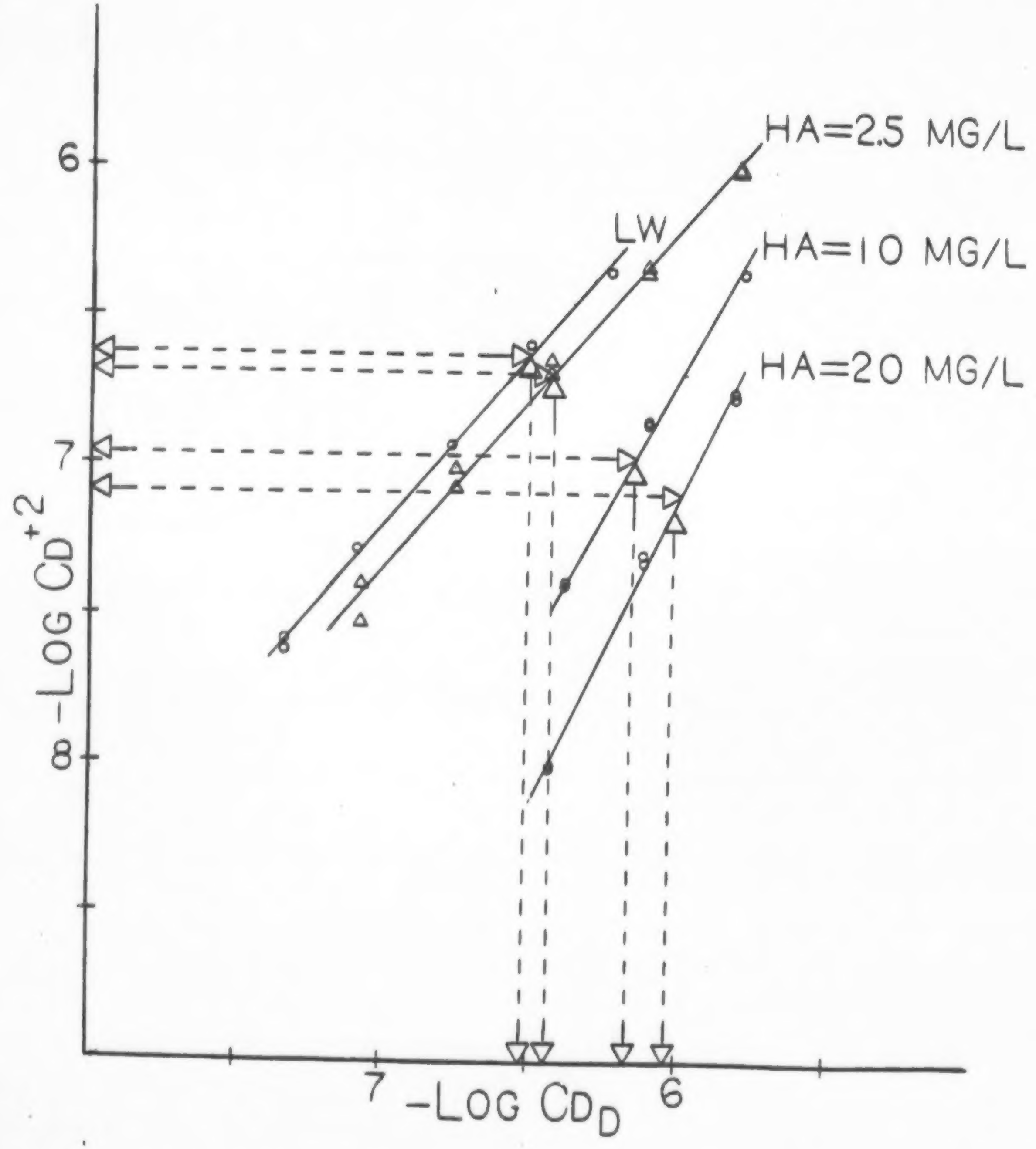

Figure 3-5B The effect of variable humic concentrations on the negative logarithms of Cd^{+2} and dissolved cadmium LC50s in Lake Superior water

tically significant decrease in the average 7 day growth weight in control water was observed. However, the organisms could still possibly be stressed at the lower humic levels which could increase the toxicities/unit concentration of toxic cadmium species. Although it is possible that some of the cadmium-humic complexes could exert toxicity directly, the mode of such action would have to be external because almost all cadmium-humic complexes appear to have molecular weights well above those which could be transported across membranes into the organism (82).

3.6 THE EFFECTS OF ALKALINITY ON COPPER TOXICITY IN LAKER SUPERIOR WATER AT AMBIENT pH

The objective of set 6 of the copper toxicity tests was to determine the effect of various levels of alkalinity on the toxicity of copper to fathead minnow larvae in Lake Superior water at ambient pH. The four test waters of set 6 consisted of Lake Superior water with the alkalinity reduced to approximately 38% of the ambient alkalinity, Lake Superior water with ambient alkalinity, and Lake Superior water with the alkalinity increased to approximately 3.5 times and to approximately 7 times the ambient alkalinity, respectively. The pH of all four test waters were maintained as close to that of the ambient pH of Lake Superior water as possible. The alkalinity of the first test water was reduced to below the ambient alkalinity by the addition of HCL. The pH of the low alkalinity water was maintained close to ambient pH by aerating the headbox with air from which a substantial portion of the CO_2 had been removed by a commercial CO_2 scrubber. The alkalinity of the third and fourth test waters was increased over that of ambient lake water by adding a ratio of $NaHCO_3$ to $KHCO_3$ which did not appear to have any effect on copper toxicity based on preliminary static toxicity tests conducted on fathead minnow larvae in waters containing various ratios of Na^+ and K^+ chlorides. The pHs of the higher alkalinity systems were maintained close to the ambient lake water pH by bubbling the headbox with CO_2.

It can be seen from Table 3-1 that large changes in alkalinity have an unexpectedly small effect on copper toxicity in terms of total or dissolved copper. Although the LC50 values in terms of total and dissolved copper for ambient Lake Superior water are almost twice that for the other three test waters, the LC50 values for the other test waters are almost identical despite large differences in the alkalinity. In contrast, the LC50 values in terms of the Cu^{+2} activity and concentration decrease monotonically with increasing alkalinity. Figure 3-6 contains four plots of the negative logarithm of the Cu^{+2} activity versus the negative logarithm of dissolved copper, one for each of the four test waters of set 6. The arrows on the Y and X axes show the relative positions of the negative logarithms of the LC50 values in terms of the Cu^{+2} activity and dissolved copper, respectively.

The results of set 6 are consistent with the results of test waters 1 and 4 of set 3 because the LC50 values in terms of total and dissolved copper were similar for those test waters even though the alkalinity of the two test waters were much different. Furthermore, just as with set 6, the LC50 values in terms of the Cu^{+2} activity and concentration decreased substantially with increasing alkalinity.

The concentrations of Cu^{+2}, $CuOH^{+}$, and $Cu(OH)_2^{\circ}$ decrease with increasing alkalinity of the test waters in set 6 whereas the concentrations of $CuCO_3^{\circ}$ and $Cu(CO_3)_2^{-2}$ increase. Therefore, equation (1-14) can be fulfilled without changes in the toxicity/unit concentration of toxic copper species occuring. However, the results for set 6 and for test waters 1 and 4 of set 3 are completely inconsistent with a model that assumes both constant toxicities/unit concentration and a much higher concentration change weighted average toxicity/unit concentration for Cu^{+2} and $CuOH^{+}$ than for $CuCO_3^{\circ}$ and $Cu(CO_3)_2^{-2}$, as will be discussed below.

It was previously shown in Section 3.3 that the toxicity/unit concentration of Cu^{+2} could be no greater than 3.5 times that of the concentration change weighted average toxicity/unit concentration of $CuCO_3^{\circ}$ and $Cu(CO_3)_2^{-2}$ if the toxicities/unit concentration remain constant in going from test water 1 of set 3 to test water 4 of set 3. Likewise, if the test waters of set 6 are compared assuming constant toxicities/unit concentration and using equation (1-17), it can be shown that the concentration change weighted average toxicity/unit concentration of Cu^{+2} and $CuOH^{+}$ is not substantially greater than that for $CuCO_3^{\circ}$ and $Cu(CO_3)_2^{-2}$. For example, if the toxicities/unit

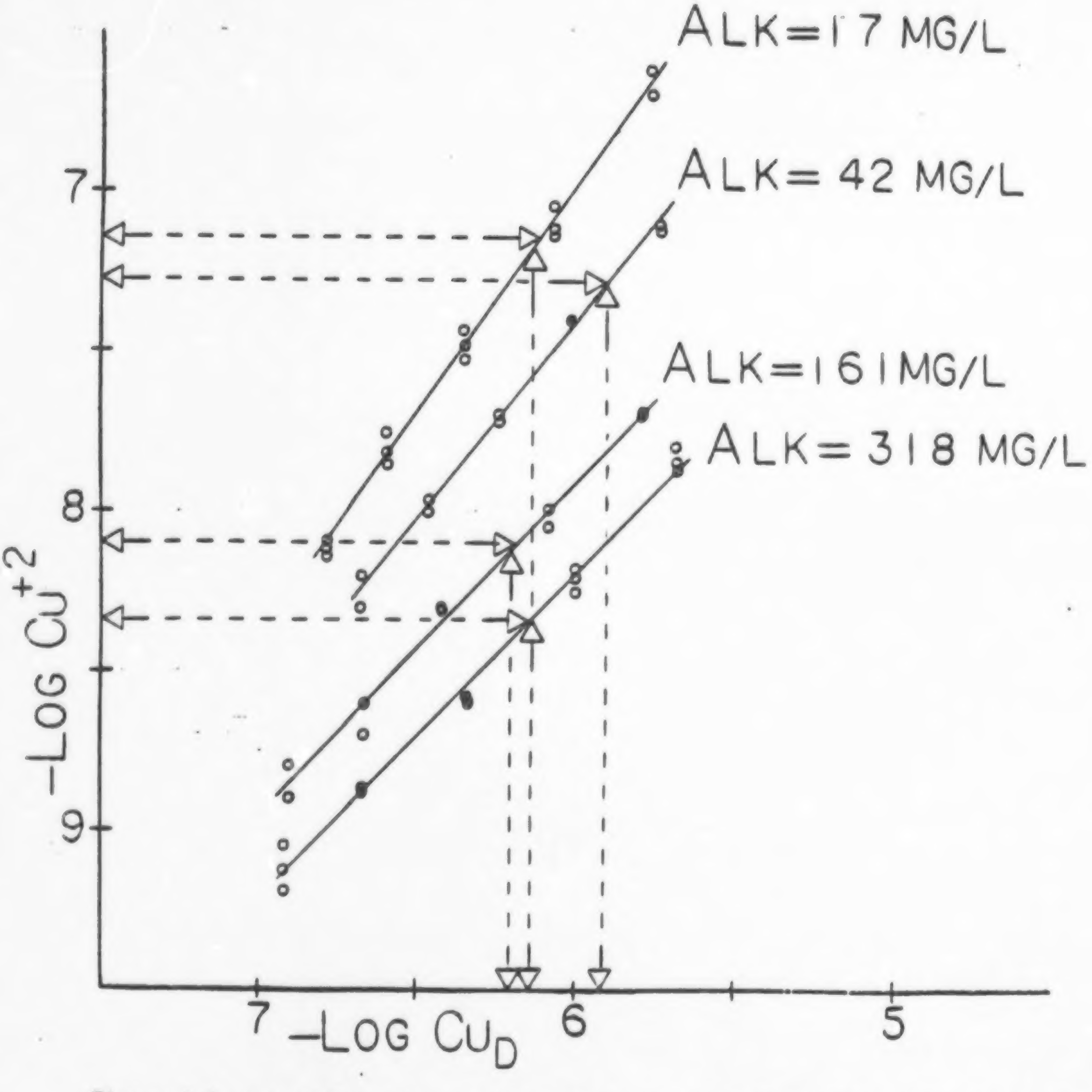

Figure 3-6 The effect of variable alkalinity on the negative logarithms of Cu^{+2} and dissolved copper LC50s in Lake Superior water at ambient pH

concentration are assumed to remain constant in going from test water 1 of set 6 to test water 4 of set 6, it can be shown from equation (1-17) that the concentration change weighted average toxicity/unit concentration of Cu^{+2} and $CuOH^{+}$ is, at most, less than 2 times greater than that for $CuCO_3^{\circ}$ and $Cu(CO_3)_2^{-2}$.

In summary, it is possible that changes in alkalinity at constant pH do not affect the toxicities/unit concentration of toxic copper species. However, if the toxicities/unit concentration of toxic copper species do remain constant for waters of different alkalinity but the same pH, it does not appear that there are large differences between the calculated concentration change weighted average toxicities/unit concentration of Cu^{+2} and $CuOH^{+}$ and that of $CuCO_3^{\circ}$ and $Cu(CO_3)_2^{-2}$. That means that the carbonate complexes could possibly contribute significantly to copper toxicity in waters with substantial alkalinity. However, recently completed static studies by Benoit and Mattson (83) have indicated that the anion of Na^{+} and K^{+} salts may have a substantial impact on the toxicity of the salt. Therefore, the ratio of $NaHCO_3$ to $KHCO_3$ that we used to raise alkalinity in these waters which was based on a no effect ratio of Na^{+} and K^{+} chloride salts, may not have been the proper ratio to use to completely nullify the opposite effects of Na^{+} and K^{+} on toxicity when added as bicarbonate salts.

3.7 FIELD STUDY ON THE NAUGATUCK RIVER, CONNECTICUT

Static 96 hour LC50s and EC50s in terms of total, dissolved and free copper (Cu^{+2}) were determined for one day old fathead minnow larvae in water samples taken from several different sites along the Naugatuck River. Static tests were run in Lake Superior water for comparative purposes, water taken from a relatively unpolluted upstream site (N1), and water taken from several downstream sites containing increasing quantities of industrial and municipal sewage effluents (N4-A, N5, N6, N7). Figure 2-2 is a map showing the location of the various sites along the Naugatuck River.

The LC50s and EC50s in terms of total, dissolved and free copper (Cu^{+2} activity) for the different site waters are listed in Table 3-4 based on measurements performed at the beginning of each test. In going from the Lake Superior water to the relatively unpolluted Naugatuck water (N1) to the increasingly polluted Naugatuck waters (N4A, N5, N6, N7), the LC50 values in terms of not only total and dissolved copper, but also the Cu^{+2} activity, generally increase. The increases in the total and dissolved copper LC50s are not surprising because the binding capacity for copper of waters containing municipal sewage effluents are generally much greater than that of similar waters containing little or no municipal sewage effluent. However, the proportional increases in the Cu^{+2} LC50 values are often also substantial. That indicates that the decrease in copper toxicity in going downstream is not just due to any increase in the binding capacity of the water because if that was the case, the increases in the Cu^{+2} LC50 values (if any) would be much smaller. Therefore, there appear to be factors introduced downstream which can not only bind copper, but reduce the toxicity of the non-bound bioavailable fraction. One of those factors may be hardness because hardness values increase monotonically in going downstream from N-1 (Hardness = 36 mg/L) to N-7 (Hardness = 90 mg/L), and because hardness has been previously shown to reduce copper toxicity.

Although the LC50 values in terms of the total, dissolved and free copper follow general trends, there are several anomalous values. In particular, the Cu^{+2} LC50 value for the N-4A water is lower than in the N-1 water even though it is much higher than for the N-1 water in all of the other downstream water samples. That anomality may have been at least partially due to possible errors in the initial determinations of the Cu^{+2} activities in (N-4A) test waters. If the Cu^{+2} LC50 value for the N-4A test water is based on Cu^{+2} determinations at the end of the 96 hour determination, it is at least comparable to the Cu^{+2} LC50 in N-1 water. The only other anomalies concern the N-7 water for which the total, dissolved and free (Cu^{+2}) copper LC50s are lower than for the N-6 water upstream. That may have been due to dilution from Great Brooke which intersects the Naugautuck River just upstream from N-7 and which may have a lower binding capacity than water from Steele Brook which intersects the Naugautuck River just upstream from N-6.

TABLE 3-4

Static 96 hour acute values for 1 day old fatheaad minnow larvae in water taken from various sites along the Naugatuck R. in Connecticut (the parentheses contain 95% confidence intervals)

	Hard and Alk in $\frac{mg}{L}$			LC50s in ug/L			EC50s in ug/L		
Water	pH	Hard	Alk	Total	Dissolved	(Cu^{+2})	Total	Dissolved	(Cu^{+2})
Lake Superior	7.74	52	55	55 (38-81)	52	1.82 (1.02-3.23)	55 (38-81)	52	1.54 (0.92-2.56)
N-1	7.44	36	38	180 (166-195)	171	3.66 (3.14-4.26)	173 (153-196)	164	3.40 (2.61-4.41)
N-4A	7.50	55	42	322 (249-415)	232	1.96^{I} 3.00^{F}	201	167	0.60
N-5	7.48	68	40	511 (439-594)	363	16.1 (12-21.7)	229	153	3.30
N-6	7.33	82	40	>998	>449	>20.1	265	133	3.46
N-7	7.28	90	43	689 (555-854)	427	14.8 (11.5-18.9)	282 (257-310)	175	3.02

The EC50s in terms of total, dissolved and free copper are also listed on Table 3-4. The EC50s are the chemical concentrations required to adversely effect the mobility of 50% of the organisms. In contrast to the LC50 values, the EC50 values in terms of total, dissolved, and free (Cu^{+2}) copper remain relatively constant in going from the N-1 water upstream to the various waters downstream (N4-A, N5, N6, N7). Again, the only major anomaly is the Cu^{+2} EC50 for the N4-A water which, as previously discussed for the Cu^{+2} LC50, may be based on erroneous activity measurements. The relative constancy of EC50 values compared to the increasing LC50 values going downstream may possibly be due to some kinetics effect that decreases the bioavailable fraction throughout the 96 hour tests (90).

The above postulate is based on the following reasoning. During a 96 hour test, the exposure of an organism to a given chemical at the gill membrane is given by

$$\text{Total Exposure} = \int_{o}^{96\ \text{hr.}} Q(t)C_{BAF}(t)dt$$

where

$Q(t)$ = flow of water across the gills as a function of time

$C_{BAF}(t)$ = concentration of the bioavailable fraction of the chemical

It should require less total exposure for an organism to develop and continue to exhibit mobility impairment than for it to be killed. If the kinetics of binding are slow enough such that the exposure of the organism is sufficient to cause mobility impairment before a substantial reduction in the bioavailable fraction occurs, but fast enough to lower the bioavailable fraction of the chemical substantially before lethal amounts of exposure occur, the EC50s should be less dependent upon the binding capacity of the water than the LC50s.

The postulate is partially but not completely supported by Cu^{+2} determinations in waters bracketing the LC50 point after the termination of the tests. The Cu^{+2} activity should be at least somewhat proportional to the bioavailable fraction. The Cu^{+2} activities in N-5 and N-6 test waters which bracket the LC50 point decreased by close to 50% and 25% during the duration of the test. Those are the two waters which show the largest increase in LC50 values from those upstream. However, the Cu^{+2} activity in one of the N-7 test waters which bracketed the LC50 increased during the test even though the LC50 for the N-7 test waters was also substantially higher than for the upstream waters N-1 and N4A.

The results of these tests, particularly for the N-4A, N-5, N-6 and N-7 test waters, should be used with caution since there was substantial algae growth in all of those test chambers at the end of the tests. Such algae growth can lead to substantial pH and D.O. fluctuations during the tests which can obviously greatly effect the bioavailable fraction and total exposure of the organisms. Such factors can effect not only acute values in terms of the free copper, but also acute values in terms of the total and dissolved copper as well. Therefore, such tests should be conducted in the future in such a way as to minimize algae growth. Possible solutions include the use of renewal or flowthrough tests instead of static tests or the use of some algacide which is non-toxic to the test organisms. The use of renewal or flowthrough tests could also have the advantage of minimizing any kinetic effects (as previously discussed) which may occur. Future Cu^{+2} activity determinations should also take into account possible absorptions of copper onto the walls of the elctrode chamber.

3.8 POSSIBLE VARIATIONS IN LARVAE SENSITIVITY TO COPPER TOXICITY OVER TIME

In developing either a chemical speciation method or a toxicity factors method for estimating LC50s, it is necessary to use data from multiple sets of toxicity tests. Therefore, it is necessary to determine if the sensitivity of larvae to copper toxicity changes over time. If the sensitivity of the larvae does change, it is then necessary to normalize all data from each set to some chosen standard test water before the data from different sets can be analyzed together to develop either a chemical speciation or toxicity factors method.

Of the six sets of copper toxicity tests run to date, five included as one test water, ambient Lake Superior water, to determine if any changes in larvae sensitivity and/or the basic diluent water occurred over time. Set 3 was the only one which did not include as a test water, ambient Lake Superior water. Figure 3-7 depicts the change in the negative logarithm of the dissolved copper and Cu^{+2} LC50s in ambient Lake Superior water between different sets as a function of the date on which each set was started. Table 3-1 and Figure 3-7 show that in going from the ambient lake water of set 1 to the ambient lake water of set 4, the dissolved copper LC50 increases monotonically by over a factor of 3 and that the Cu^{+2} LC50 increases monotonically by over a factor of 6. The chemical speciation calculations in Table (3-2) show that the calculated concentrations at the LC50 point of all of the other major inorganic copper species greatly increase as well and that the calculated concentration of the theoretical copper-organic fraction increases despite a proportional decrease. Therefore, in order for equation (1-14) to hold in going from the ambient lake water in set 1 to the ambient lake water in set 4, it would be necessary for the toxicity/unit concentration of one or more toxic copper species to decrease, assuming that the calculation showing the concentration of the copper-organic fraction increasing is at least qualitatively correct. Of course, as always, there is a chance that the concentration of the copper-organic fraction actually decreases in going from the set 1 to the set 4 ambient lake water because the calculation of the copper-organic fraction is subject to large error. If that was the case, it would be theoretically possible for equation (1-14) to be fulfilled without any change in the toxicities/unit concentration. However, it is unlikely that any decrease in the concentration of the copper-organic fraction would be large enough to offset the relatively large increases in the concentrations of all of the major inorganic copper species.

The above discussion indicates that the sensitivity of fathead minnow larvae to copper toxicity changes over time, and that such changes may be reflected in apparent changes in the toxicity/unit concentration of one or more toxic copper species. If this is the case, it will probably be necessary in the future to run one ambient lake test water per each set of tests to use as a reference water, to designate one such ambient lake test water as the

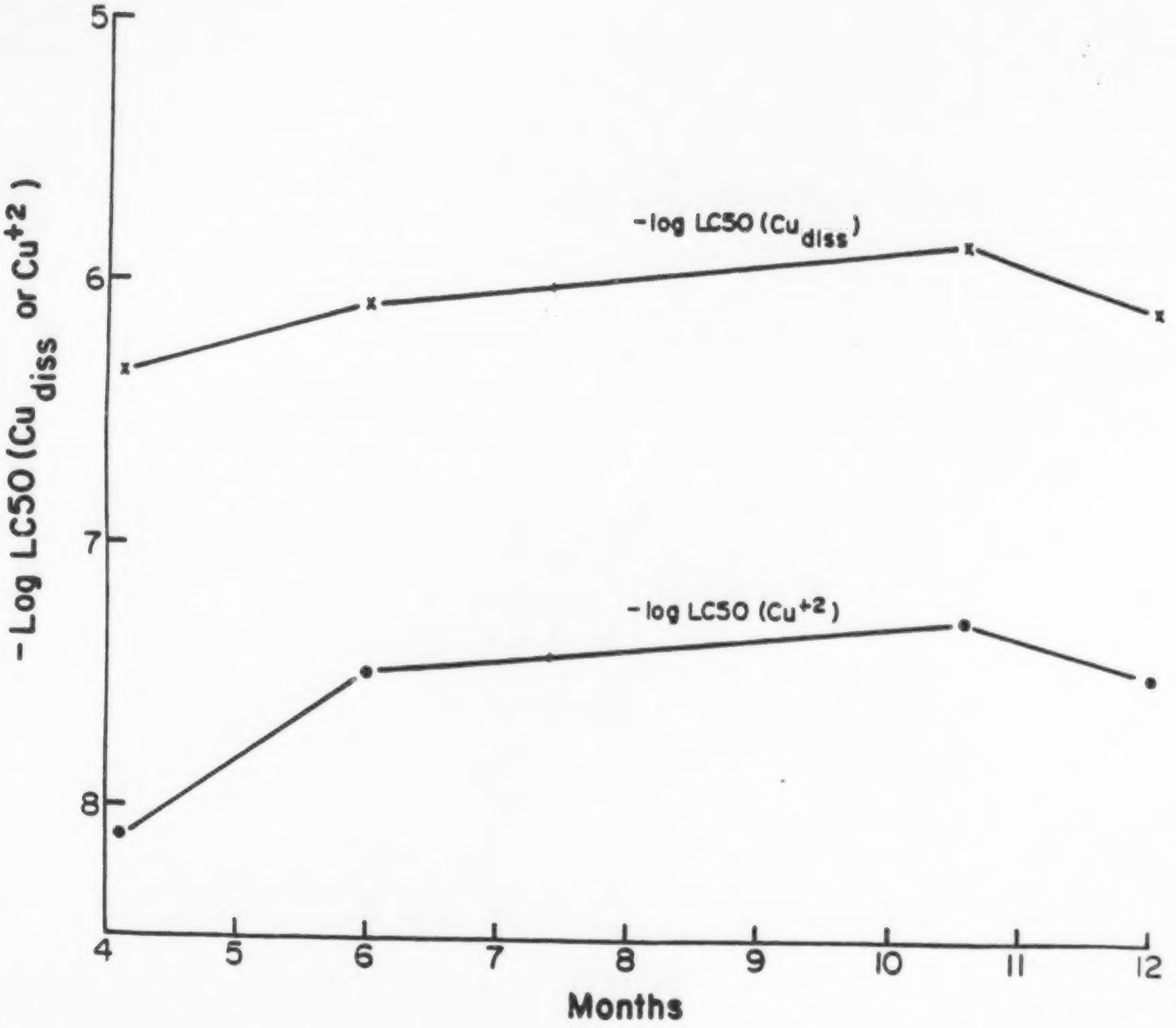

Figure 3-7. The variation in the negative logarithms of Cu^{+2} and dissolved copper LC50s in ambient Lake Superior water between different sets over time

standard test water, and to normalize the data from each set to that of the standard water by multiplying all Cu^{+2} concentration data in the set by the ratio of the Cu^{+2} LC50 in the reference water for the set to the Cu^{+2} LC50 in the standard water.

3.9 DIFFERENCES BETWEEN STEADY STATE AND EQUILIBRIUM CONCENTRATIONS

The accuracy of equations (1-1) to (1-7) for calculating the steady state concentrations of copper hydroxy and copper carbonate species from the steady state activity of Cu^{+2} will depend to a large extent on how close those chemical species are to being in equilibrium with the Cu^{+2} at steady state, regardless of how far the Cu^{+2} steady state activity may be from the Cu^{+2} equilibrium activity. The reason is that the equations are based on an assumption of equilibrium between the given species (e.g., $CuOH^{+}$) and Cu^{+2}, not upon the assumption that the Cu^{+2} is in equilibrium with the entire system or that the entire system is at equilibrium. Therefore, any difference between the steady state and equilibrium Cu^{+2} activity should represent only the worst possible error in the calculation of the steady state concentrations of the copper hydroxy and copper carbonate species.

Table 3-5 contains ratios of equilibrium to steady state Cu^{+2} activities for duplicate sets of four waters representing the various types of waters considered in the six sets of copper toxicity tests; ambient Lake Superior water, lake water with elevated alkalinity and pH, lake water with added humics, and lake water with lowered alkalinity and pH. The table lists by column the type of water, the duration of the experiment which was generally longer than the time required to reach equilibrium, the ratio of the Cu^{+2} activity in the Teflon bottle at the end of the experiment to the Cu^{+2} activity in the flow-through chamber before the transfer to the Teflon bottle, the ratio of the equilibrium Cu^{+2} activity to the steady state Cu^{+2} activity, and the difference between the pH in the flow-through chamber before transfer to the Teflon bottle and the pH in the Teflon bottle at the end of the experiment. Absorption is taken into account by computing the ratio of Cu^{+2} activities listed in column 5 from the product of the ratios listed in columns 3 and 4.

TABLE 3-5

Comparison of Equilibrium Cu^{+2} Activities to Steady State Cu^{+2} Activities

	Time	$\frac{(Cu^{+2})_{Bottle}}{(Cu^{+2})_{Flow}}$	$\frac{(Cu_T)_{Flow}}{(Cu_T)_{Bottle}}$	$\frac{(Cu^{+2})_{Equil}}{(Cu^{+2})_{ss}}$	$(pH)_{Flow} - (pH)_{Bottle}$
Ambient Lake H_2O	5 hrs	0.95	1.03	0.98	0.04
Ambient Lake H_2O	7 hrs	0.88	1.02	0.90	0.03
Alk = , pH =	7 hrs, O.N.[b]	0.89	0.96	0.86	-0.03
Alk = , pH =	7 hrs	0.91	--[a]	0.91	0.01
Humics (2.5 mg/L TOC)	12 hrs, O.N.[b]	0.78, 0.78[b]	1.06[b]	0.83, 0.83[b]	0.11
Humics (2.5 mg/L TOC)	10 hrs	0.79	0.98	0.77	-0.19
Alk = , pH =	10 hrs, O.N.[b]	0.69, 0.68[b]	1.12	0.78, 0.76[b]	0.08
Alk = , pH -	7 hrs, O.N.[b]	0.79, 0.70[b]	1.15[b]	0.80, 0.80	0.02

[a] Sample misplaced

[b] Overnight

The equilibrium to steady state Cu^{+2} ratios listed in column 5 of Table 3-5 show that the difference between the equilibrium and steady state Cu^{+2} activities in ambient lake water and in lake water with elevated alkalinity and pH is at most less than 10% and 15%, respectively. The results are consistent with the good agreement in most cases for waters with elevated alkalinity and/or pH between the measured dissolved copper and the sum of calculated concentrations of major inorganic species based on the use of measured steady state Cu^{+2} activities in equations (1-1) to (1-7).

The ratios of equilibrium to steady state Cu^{+2} activities listed in column 5 of Table 3-5 show that the difference between the equilibrium and steady state Cu^{+2} activities in lake water with added humics, and in lake water with lowered alkalinity and pH is, at most, less than 25%. That is somewhat greater than the difference in ambient lake water or in lake water with elevated alkalinity and pH. Actual differences between equilibrium and steady state Cu^{+2} activities of greater than 15% could conceivably introduce some significant error in using equilibrium equations (1-1) to (1-7) to calculate steady state concentrations of chemical species from the steady state Cu^{+2} activity. However, as stated previously, the differences indicated by Table 3-5 represent only worse case situations because the species whose concentrations are calculated from equation (1-1) to (1-7), may be closer to equilibrium with Cu^{+2} than the Cu^{+2} is to equilibrium with the system as a whole.

3.10 THEORETICAL COPPER-ORGANIC COMPLEXATION BASED ON THE SLOPES OF THE ELECTRODE RESPONSE

The accuracy and utility of using the cupric ion electrode to determine Cu^{+2} activities in natural waters has been questioned by several groups (46, 74, 84-86). Ions which appear to interfere with the electrode include Ag^{+}(72), Hg^{+2}(72), Fe^{+3}(72, 84) and Cl^{-}(72, 74, 84). In addition, the electrode is susceptible to oxidative dissolution of Cu^{+2} in aqueous solution (particularly in acidic solutions) and apparently becomes slowly oxidized when stored in air (73-75). Humics may also possibly interfere with the electrode either by coating the membrane (86) or by changing the standard potential of the electrode (46).

Although interferences by Ag^+, Hg^{+2}, or Fe^{+3} are possible in some natural waters, the concentration of all of those cations in Lake Superior water, which was used as the diluent water for all in lab toxicity tests, are relatively low (57) and therefore should not have posed a significant problem. The use of a double junction reference electrode should have protected the solutions in which measurements were made from Ag^+ contamination. Although Cl^- concentrations in Lake Superior water are also low, several tests within set 1 of the toxicity experiments involved adding $CaCl_2$, $MgCl_2$ or NaCl to a nominal concentration of $4x10^{-3}M\ Cl^-$. However, those levels of Cl^- are still well below (eg > 10x less) those levels reportedly required to adversely affect electrode performance (72, 87-89). Although the Cu^{+2} determination in the NaCl solution appeared to be too high, the Cl^- concentration in that solution was less than in the $CaCl_2$ or $MgCl_2$ solutions where the Cu^{+2} determinations were in close agreement with computer estimates.

Although we observed evidence of Cu^{+2} dissolution off the membrane, it did not appear to be a significant problem except in solutions with a pH below 6. Furthermore, the Cu^{+2} activities in the lab flow through chambers were measured in a flow-through electrode chamber designed to carry solution exposed to the electrode rapidly from the electrode and to continuously impact the electrode membrane with fresh solution. Therefore, it is unlikely that the electrode measured significant quantities of any Cu^{+2} built up from dissolution off the electrode membrane. The electrode was routinely polished daily and was polished more frequently if measurements within duplicate tanks on the same day varied by more than 2mV. Therefore, it is also unlikely that any tarnishing of the electrode membrane through solution oxidation, air oxidation, or coating by humics significantly affected Cu^{+2} determinations. The suggestion that humics could possibly substantially alter the standard potential of the electrode pair is difficult to prove or disprove and has not, as yet, been confirmed or disproven by other groups. Finally, we saw little evidence of the cupric ion electrode responding to either copper hydroxy or copper carbonate complexes as was reported by Wagemann 1980 (85). Although the Cu^{+2} determination in the high alkalinity and pH water of set 3 appeared to be high, measurements in other high alkalinity and/or pH waters appear to agree closely with computer estimated values.

If the potential E of a cupric ion electrode/double junction reference electrode pair is plotted versus the log of the dissolved copper, the slope of the plot at any given point can be shown, assuming Nernstian behavior to be given by

$$\frac{dE}{d\log Cu_{DISS}} = N - N(CU^{+2})\,\frac{dD}{dCu_{DISS}} \qquad (3\text{-}1)$$

where

N = Nerstian slope

$D = Cu_{DISS}/Cu^{+2}$

If the dissolved copper consists only of Cu^{+2}, copper hydroxy and copper carbonate species, D will only be a function of pH and alkalinity so that

$$\frac{dE}{d\log Cu_{DISS}} = N - N(Cu^{+2})\left[\left(\frac{\partial D}{\partial pH}\right)_{Alk}\frac{dpH}{dCu_{DISS}} + \left(\frac{\partial D}{\partial Alk}\right)_{pH}\frac{dAlk}{dCu_{DISS}}\right] \qquad (3\text{-}2)$$

Since $\frac{dpH}{dCu_{DISS}}$ and $\frac{dAlk}{dCu_{DISS}}$ are both negative and $\left(\frac{\partial D}{\partial pH}\right)_{Alk}$ and $\left(\frac{\partial D}{\partial Alk}\right)_{pH}$ are both positive, the grouped expression within the brackets of the second term of equation (3-2) is negative and the overall second term of equation (3-2) is positive. Therefore, the slope of the electrode response, even in organic free water, is expected to be greater than Nernstian, provided that the added copper is sufficient to significantly decrease the pH and/or alkalinity of the water.

Table 3-6 lists by column for each of the test waters for each of the six sets of copper toxicity experiments the chemical substituents added to the test waters, the pH, alkalinity and hardness of the test waters, the computed free copper (Cu^{+2} concentration) percentages of dissolved copper based on electrode determinations of Cu^{+2} activities, the REDEQL computed free copper percentages based on the dissolved copper assuming no organic complexation of copper, the ratio of the computed free copper percentages based on electrode determinations to those based on REDEQL assuming no organic complexation, the percentage difference between dissolved copper and the sum of all major

TABLE 3-6

Comparison of Computed Free Copper Percentage of Dissolved Copper
at the LC50 Point Based on Electrode Determinations to REDEQL
Computed Free Copper Percentages Based on Inorganic Speciation Alone
for the Six Sets of Copper Toxicity Tests

	pH	Alk	Hard	% Cu^{+2} Electrode	% Cu^{+2} REDEQL	Ratio	Tests % Cu-Org	Slope mV/Log Unit
Set 1 - 4/83 (Ca^{+2}, Mg^{+2}, Na^+ Effects)								
Ambient Hardness	8.13	42.6	47.0	2.03	3.26	0.62	46.5	40.5
$2x10^{-3}$M $CaCl_2$	8.05	43.1	243	4.96	4.88	1.02	14.4	33.7
$2x10^{-3}$M $MgCl_2$	8.06	43.3	255	4.10	4.83	0.85	26.7	36.5
$2x10^{-3}$M NaCl	8.12	43.3	47.2	5.30	3.60	1.47	0	35.9
Set 2 - 6/83 (pH Effects at Ambient Alkalinity)								
CO_2 Bubbling	6.58	48.9	49.2	13.5	51.8	0.26	77.3	42.1
CO_2 Bubbling	7.35	44.5	46.2	10.0	16.5	0.61	47.9	39.1
Ambient pH	8.06	42.8	45.1	3.98	3.82	1.04	11.6	37.5
NaOH Added	8.72	47.4	45.2	0.75	0.75	1.00	11.7	34.1
Set 3 - 7/83 (pH Effects at 3X Ambient Alkalinity)								
$(Na^+,K^+)HCO_3^-$, CO_2	7.15	155	46.2	11.2	9.91	1.13	2.02	27.6
$(Na^+,K^+)HCO_3^-$, CO_2	7.94	148	45.0	1.94	1.77	1.10	5.98	28.0
$(Na^+,K^+)HCO_3^-$	8.56	150	45.0	0.58	0.38	1.52	0	30.3
Hcl Added	7.16	26.4	45.0	20.4	32.2	0.63	43.5	33.8

TABLE 3-6 (continued)

	pH	Alk	Hard	% Cu^{+2} Electrode	% Cu^{+2} REDEQL	Ratio	Tests % Cu-Org	Slope mV/Log Unit
Set 4 - 10/83 (Effects of Clay, Humics)								
Ambient Lake Water	8.03	40.8	42.5	3.74	4.24	0.88	24.1	38.1
Suspended Clay (70 NTL)	8.22	46.5	48.9	2.01	2.46	0.82	29.7	39.3
Humics (5 mg/L TOC)	8.00	44.2	45.5	0.50	4.20	0.12	90.1	60.7
Clay and Humics	8.22	48.5	46.8	0.47	2.36	0.20	83.2	56.4
Set 5 - 11/83 (Effects of Variable Humics Concentrations)								
Ambient Lake Water	8.02	43.0	45.0	4.32	4.17	1.04	11.3	36.0
Humics (1.25 mg/L TOC)	8.00	42.2	45.0	2.19	4.36	0.50	57.4	45.1
Humics (2.50 mg/L TOC)	8.00	43.8	45.5	1.13	4.28	0.26	77.6	54.3
Humics (5.0 mg/L TOC)	8.02	42.8	45.0	0.47	4.17	0.11	90.4	57.0
Set 6 - 5/84 Alkalinity Effects at Ambient pH)								
HCl Added, CO_2 Removed	7.84	17.0	44.0	11.5	12.0	0.96	18.0	41.0
Ambient lake Water	7.93	42.0	44.0	5.02	5.20	0.97	17.5	35.3
$(Na^+,K^+)HCO_3^-$	8.00	161	44.0	1.71	1.49	1.14	4.1	30.1
$(Na^+,K^+)HCO_3^-$	8.00	318	45.0	0.90	0.79	1.14	3.8	29.6

inorganic copper concentrations based on electrode determinations of Cu^{+2} activities (designated % Cu-Org), and the linear regression derived slopes of the electrode response.

The slopes of the electrode response listed in Table 3-6 are all far greater than Nernstian except the ones listed for waters with higher than ambient lake water alkalinity. The slopes of the electrode response in waters with higher than ambient alkalinity are closer to Nernstian presumably because the added buffering capacity makes $\frac{dpH}{dCu_{DISS}}$ and $\frac{dAlk}{dCu_{DISS}}$ small so that the slope given by equation (3-2) reduces to approximately Nernstian. However, even though greater than Nerstian slopes are expected in test waters run at the lower alkalinities, the magnitude of the observed greater than Nernstian slopes cannot be explained alone by the relatively small and often negligible observed decreases in pH and alkalinity with increasing dissolved copper within the low upper range of copper used for our toxicity tests. Therefore, we have postulated the existence of one or more organic ligands which have a relatively strong affinity for Cu^{+2}, but which have low activities such that complexing with Cu^{+2} substantially lowers the ligand activity. In such a case, the slope of the electrode response would be given by .

$$\frac{dE}{dlogCu_{DISS}} = N-N(Cu^{+2}) \left[\left(\frac{\partial D}{\partial pH}\right)_{Alk} \frac{dpH}{dCu_{DISS}} + \left(\frac{\partial D}{\partial Alk}\right)_{pH} \frac{dAlk}{dCu_{DISS}} + \left(\frac{\partial D}{\partial L_i}\right)_{AlkpH} \frac{dL_i}{dCu_{DISS}} \right]$$

where

L = activity of i^{th} ligand

and the slope would depend not only on decreases in the pH and alkalinity, but also decreases in organic ligand activities with increasing dissolved copper.

The ratios of the electrode determined free copper percentages to the REDEQL determined free copper percentages at the LC50 point, assuming no organic complexation are listed in column 7 of Table 3-5. The ratios for 12 of the 24 test waters of the six sets of copper toxicity experiments are within the range 0.85-1.15. Of the 12 test waters outside of this range, six exhibit very low ratios and contain added humics and/or clay which can complex substantial amounts of copper beyond that predicted by REDEQL based on inorganic speciation alone. Of the remaining six test waters outside the range, one is the ambient lake water of set 1, and three are waters with lower than ambient pH and/or alkalinity all of which exhibit low ratios. Copper-organic complexation could possibly be more extensive in waters with lower alkalinity and/or pH due to a reduction in competition between the organic ligands, OH^-, and CO_3^{-2} for Cu^{+2} even though lower pH waters may have increased competition between Cu^{+2} and H^+ for organic ligands. The two remaining waters (the NaCl water of set 1 and the high alkalinity and pH water of set 3) had high ratios of approximately 1.53, which we cannot explain.

The non-humic waters listed in Table 3-6 with the greatest non-Nernstian slopes also generally have the lowest electrode/REDEQL free copper percentage ratios and the highest theoretical copper-organic fraction percentages. However, those observed correlations are the result of the affects of a steeper than Nernstian slope on related computations, and would be observed regardless of whether or not a copper-organic fraction contributed significantly to the non-Nernstian slope. Therefore, we attempted to determine if a significant copper-organic fraction existed in Lake Superior water despite the low organic content of the water. In doing so, we performed two sets of titration experiments.

The first set of titrations was designed to observe the effects of reduced organic content on the greater than Nernstian slope of the electrode response. The first set of experiments consisted of $10^{-3}M\ Cu(NO_3)^2$ titrations of Lake Superior water, UV irradiated Lake Superior water, Burdick and Jackson HPLC grade water, UV irradiated Burdick and Jackson water, and Burdick and Jackson water passed through a HPLC column. Figure 3-8 contains plots of the negative logarithm of the Cu^{+2} activity versus the negative logarithm of the total copper for the five titrations comprising the first set.

Figure 3-8 shows that it is possible for waters with supposedly lower organic contents than Lake Superior water such as HPLC grade Burdick and Jackson water to exhibit even greater non-Nernstian slopes and lower Cu^{+2}/Cu_T

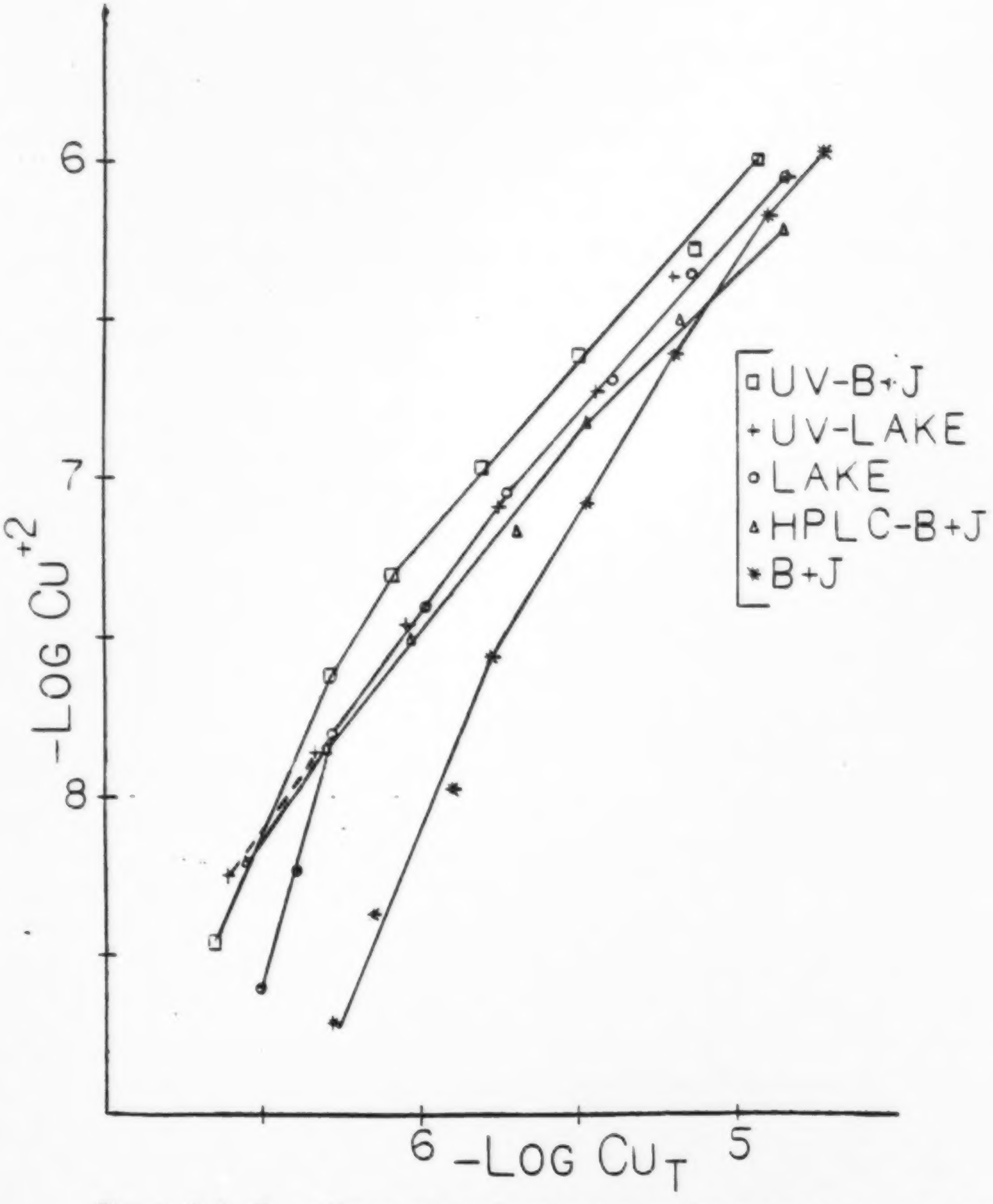

Figure 3-8 The effects of UV irradiation and/or HPLC clean up on copper titrations of Lake Superior water and Burdick + Jackson water

ratios at each point of a titration curve. It is unclear whether the addition of $NaHCO_3$ to the Burdick and Jackson water to increase the alkalinity to that of lake water also introduced organic contamination to the water. However, the organic content of the Burdick and Jackson water even after the addition of $NaHCO_3$ should have been less than the average 1ppm TOC of Lake Superior water since the amount of $NaHCO_3$ added ($1x10^{-3}M$) was small. The large, steeper than Nernstian slope and relatively low Cu^{+2}/Cu_T ratios for the Burdick and Jackson water titration could have been due to some factor other than copper-organic complexation such as electrode interference. However, it is unlikely since Figure 3-8 shows that UV irradiation decreases the slope of the Burdick and Jackson water to that comparable to lake water, and increases the free Cu^{+2}/Cu_T ratios to above those for lake water presumably through photochemical degradation of organics. Passing the Burdick and Jackson water down a C-18 reverse phase HPLC column to remove organics also decreases the slope of the titration curve and increases Cu^{+2}/Cu_T ratios, although not to the extent that UV irradiation did as can be seen in Figure 3-8.

Although it appears that copper-organic complexation is responsible for most of the non-Nernstian characteristics of the Burdick and Jackson water titration curve, it is not certain. That is because neither UV irradiation nor HPLC results in linear-Nernstian titration curves despite the elimination of pH change contributions to the non-Nernstian behavior [by performing constant pH (8.00) titrations] and the negligible contribution of alkalinity changes (as demonstrated by the small differences between pre-titration and post-titration alkalinities). Nevertheless, both UV irradiation and HPLC greatly reduce the non-Nernstian characteristics of the Burdick and Jackson water titration curve so that the failure to produce completely Nernstian curves may be due to insufficient organic removal.

The above discussion showed that it might be possible for waters with comparable or even lower organic content to exhibit even greater non-Nernstian behavior than Lake Superior water through copper-organic complexation. However, figure 3-8 shows that the UV irradiation of Lake Superior water had virtually no effect on the titration curve except at the lowest section of the curve. That is contrary to what would be expected if substantial amounts of copper-organic complexation occurred in Lake Superior water. Unfortunately, the UV system used was not set up for continuous UV irradiation, so UV irradiation was performed during three successive siphonings of the water through the photochemical reactor cell. Since there was no operational TOC analyzer available, it was not possible to determine if the resultant duration and intensity of the UV irradiation was sufficient to appreciably reduce the organic content of the Lake Superior water. Although by using the same procedure and equipment, UV irradiation did appear to appreciably reduce the organic content of the Burdick and Jackson water, the organics in Lake Superior water, particularly the naturally occurring higher molecular weight ones, may be more resistant to UV degradation. Therefore, it is unclear whether the failure of UV irradiation to appreciably affect the titration curve for Lake Superior water was due to an insufficient reduction in the organic content of the water, or to factors other than copper-organic complexation such as electrode interferences being primarily responsible for the non-Nernstian characteristics of the titration curve.

The failure of the first set of titrations to conclusively demonstrate the presence of significant copper-organic complexation in Lake Superior water led to the performance of a second set of six titrations designed to observe the effects of ionic strength, cations competitive with Cu^{+2} for organic ligands such as Ca^{+2} and Zn^{+2}, alkalinity and pH on the titration curves of Lake Superior water. The second set of titrations consisted of 10^{-3} $Cu(NO^3)_2$ titrations of Lake Superior water and seperate Lake Superior waters with the following nominal concentrations: $2x10^{-3}Ca(NO_3)_2$, $10^{-4}M\ Zn(NO_3)_2$, $6x10^{-3}M\ NaNO_3$ and $4x10^{-3}M\ NaHCO_3$. Titrations of the $4x10^{-3}M\ NaHCO_3$ waters were performed at pHs of 8.00 and 8.50. Figure 3-9 contains plots of the negative logarithm of Cu^{+2} versus the negative logarithm of total copper for each of the six titrations of the second set.

The plots in figure 3-9 are consistent with the postulate that significant copper-organic complexation does occur in Lake Superior water. For example, the slopes of the titration curves for lake water containing $10^{-4}M\ Zn(NO_3)_2$ or $2x10^{-3}M\ Ca(NO_3)_2$ are somewhat closer to Nerstian (e.g., for the type of plots in figure 3-8 and 3-9, a Nernstian curve is linear with a slope of 1)

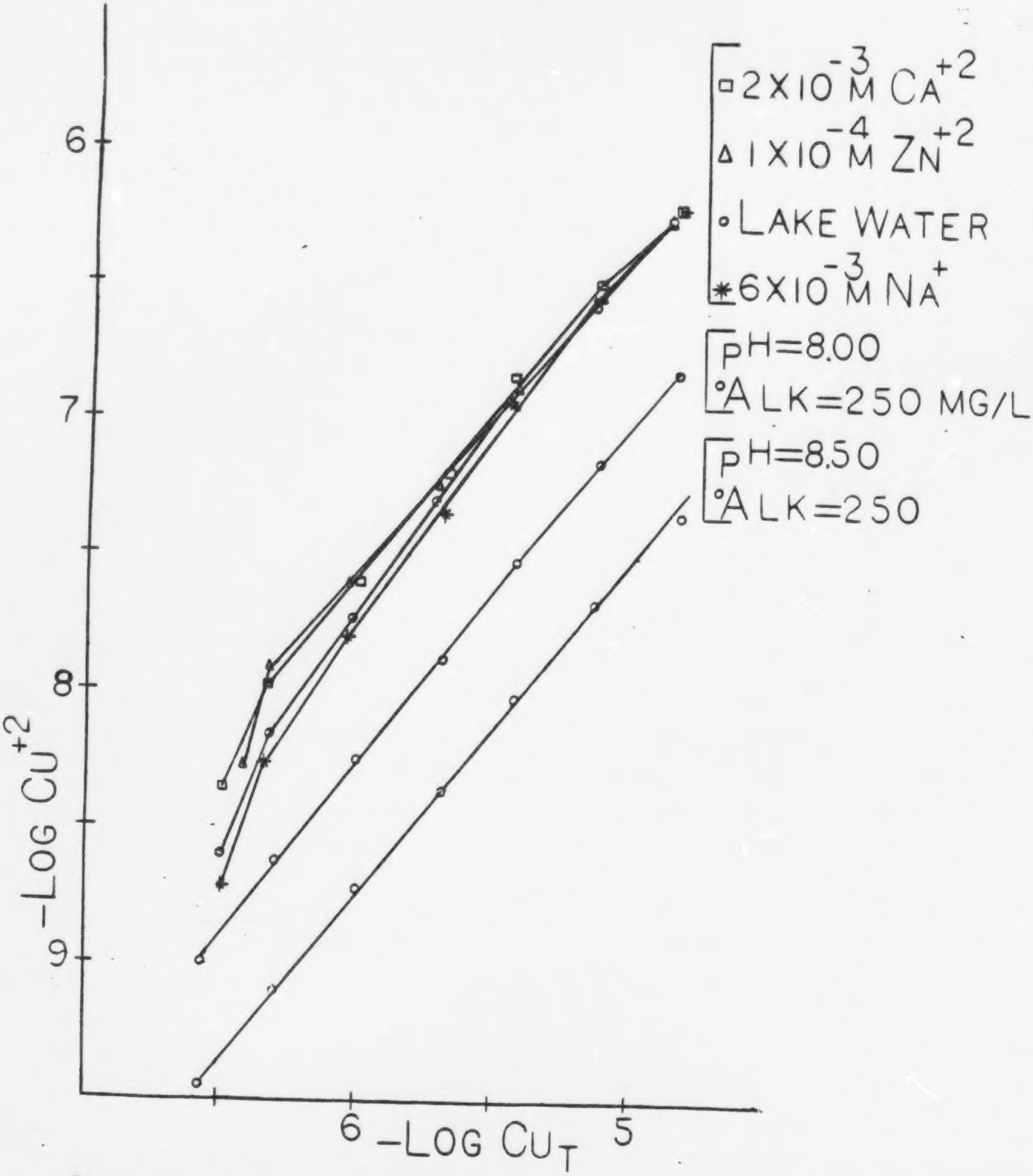

Figure 3-9 The effects of Ca^{+2}, Zn^{+2}, Na^{+}, pH and alkalinity on copper titrations of Lake Superior water

than Lake Superior water, although both curves are curvilinear. The titration curves for lake water to which 4×10^{-3}M $NaHCO_3$ was added were actually linear with slopes much closer to Nernstian than Lake Superior water. The effects of Ca^{+2}, Zn^{+2} and $NaHCO_3$ on titration curves for Lake Superior water cannot be due to increases in the ionic strength because the addition of 6×10^{-3}M $NaNO_3$ has very little effect on the titration curve as can be seen in figure 3-9. The observation that $Ca(NO_3)_2$ and $Zn(NO_3)_2$ affect the slope of the titration curves whereas $NaNO_3$ does not supports the postulate that significant copper-organic complexation is present in Lake Superior water. The reason is that Ca^{+2} and Zn^{+2} both exhibit much higher affinities for organics than does Na^+ and therefore, could conceivably effectively compete with Cu^{+2} for organic ligands.

The effect of $NaHCO_3$ and the associated increase in alkalinity on the slope of the titration curves is probably due to increased competition of CO_3^{-2} with organic ligands for Cu^{+2}, which may lead to a reduction in copper-organic complexation. Likewise, the increase in pH to 8.50 probably leads to increased competition between OH^- and organic ligands for Cu^{+2}. Table 3-7 shows that relatively good agreement can be obtained between computed free copper percentages based on electrode determinations and computed free copper percentages based on REDEQL for the Lake Sueprior water titration, the 4×10^{-3}M $NaHCO_3$ lake water titration at pH 8.00 and the 4×10^{-3}M $NaHCO_3$ lake water titration at pH 8.50 if variable glycine concentrations are input into the REDEQL program. The variable glycine concentrations are indicated in Table 3-7 footnotes, and all are either comparable or well below that which would correspond to the reported average organic nitrogen (0.1 ppm) and TOC (1 ppm) for Lake Superior water (92, 57). The fact that decreasing assumed glycine concentrations with increasing copper are required to maintain reasonable agreement between electrode and REDEQL computations may correspond to the gradual saturation of binding sites and/or to increased negative interactions between bound and unbound sites of copper binding organics in Lake Superior water. Although the use of glycine is not meant to accurately model the binding capacity of Lake Superior water, it does show that an organic with a somewhat intermediate binding affinity for copper compared to many other organic ligands and at concentrations at or well below that corresponding to

TABLE 3-7

Comparison of Computed Free Copper Percentage of Total Copper Based on Electrode Determinations to REDEQL Computed Free Copper Percentages Based on Assumed Variable Glycine Concentrations

	-Log Cu_T	% Cu^{+2} Electrode	% Cu^{+2} REDEQL	Ratio
Ambient Lake Water				
	6.49	0.90	4.19[a]	0.21
			0.73[b]	1.23
	6.33	1.75	4.19[a]	0.42
			1.84[c]	0.95
	6.03	2.36	4.19[a]	0.56
			2.38[d]	0.99
	5.73	3.10	4.19[a]	0.74
			2.95[e]	1.05
	5.47	4.09	4.19[a]	0.98
			3.65[f]	1.12
	5.15	4.39	4.19[a]	1.05
			3.00[g]	1.10
	4.88	4.92	4.19[a]	1.17
			4.09[h]	1.20
$4x10^{-3}$M $NaHCO_3$, pH = 8.00				
	6.57	0.36	0.47	0.77
	6.29	0.47	0.70	0.67
	6.01	0.58	0.79	0.73
	5.69	0.65	0.84	0.77
	5.43	0.81	0.92	0.88
	5.13	0.93	0.94	0.99
	4.83	1.05	0.94	1.12
$4x10^{-3}$M $NaHCO_3$, pH = 8.50				
	6.57	0.13	0.10	1.30
	6.30	0.16	0.19	0.84
	6.01	0.19	0.22	0.86
	5.69	0.21	0.24	0.88
	5.14	0.30	0.26	1.15
	4.83	0.30	0.24	1.11

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[a] Glycine Concentration = 0

[b] - LOG [Gly] = 5.10

[c] - LOG [Gly] = 5.40

[d] - LOG [Gly] = 5.58

[e] - LOG [Gly] = 5.70

[f] - LOG [Gly] = 6.00

[g] - LOG [Gly] = 6.30

[h] - LOG [Gly] = 6.60

the average organic nitrogen and TOC of Lake Superior water, can bind substantial amounts of copper. Furthermore, the good agreement between electrode and REDEQL computations in Table 3-7 for the 4×10^{-3}M $NaHCO_3$, particularly the one at pH 8.50 shows that increased alkalinity and/or pH can effectively modify non-Nernstian characteristics caused by copper-organic complexation (in this case, the assumed copper-glycine complexation).

Although neither the set 1 nor set 2 titrations conclusively show the presence of significant copper-organic complexation in Lake Superior water, they do support that postulate with the exception of the failure of UV irradiation to significantly affect the Lake Superior titration curve, which may have been due to an insufficient reduction of organic content.

3.11 MULTIPLE LINEAR REGRESSIONS

Despite apparent variations in the toxicity/unit concentration of toxic copper species, an attempt was made to compute the toxicities/unit concentration of the five major inorganic copper species (T_1,T_2,T_3,T_4,T_5) and an average toxicity/unit concentration for the copper-organic fraction (T_6) by regressing $Y = 1/Cu^{+2}{}_{LC50}$ on X_1, X_2, . . . X_6 of equation (1-10) where X_1, X_2, . . . X_6 are related to the computed concentrations at the LC50 point of the various copper species by the definitions given for equation (1-10). The first attempt was made using the calculated concentrations of all five major inorganic species and the theoretical copper-organic fraction for 14 of the 24 test waters. Of the six toxicities/unit concentration calculated, four were statistically different from zero at $p = 0.05$. However, the calculated toxicities/unit concentration of $CuOH^+$ and $Cu(CO_3)_2{}^{-2}$, which were two of the four statistically different from zero, were negative which has no physical meaning.

The multiple linear regression was then repeated by regressing Y on only those variables related to inorganic species and using data only from the 9 of the 14 test waters used originally which had negligible theoretical copper--organic fractions. Although the resulting regression equation accounted for greater than 98% of the variance in Y, only two of the five computed toxicities/unit concentration were statistically different from zero at $p = 0.05$. Furthermore, of the two statistically significant toxicities/ unit concentration, the one for $Cu(CO_3)_2^{-2}$ was again negative.

The unsuccessful attempt to compute toxicities/unit concentration from multiple linear regression may be primarily due to the variation and dependence of toxicities/unit concentration on such factors as pH, and perhaps time, as reflected by the variation in larvae sensitivity with time depicted in Figure 3-7.

4. CONCLUSIONS AND RECOMMENDATIONS

In this chapter, our conclusions with respect to the feasibility of developing both a chemical speciation method and a toxicity factors method for predicting copper toxicity in site waters will be presented along with recommendations for future research. However, a summary of discussions on the apparent dependence of toxicities/unit concentration on the values of general water quality parameters and/or time, and on the possible presence of toxic copper-organic complexes in Lake Superior water will be presented first.

4.1 THE APPARENT DEPENDENCE OF TOXICITIES/UNIT CONCENTRATION ON THE VALUES OF GENERAL WATER QUALITY PARAMETERS AND/OR TIME

An analysis of the results of set 1 of the copper toxicity tests in Section 3.1 indicated that the toxicities/unit concentration of at least some toxic copper species probably decrease with increasing hardness. The increase in the dissolved copper LC50 in going from ambient lake water to the 2×10^{-3} M $CaCl_2$ water cannot be contributed to by any proportional changes in Cu^{+2} or any other major inorganic copper species because the proportion of all such species increase (see Table 3-3). The increase in the dissolved copper LC50 may be partially due to a decrease in the proportion of a possibly toxic copper-organic fraction perhaps caused by competition between Ca^{+2} and Cu^{+2} for organic ligands. However, it appears likely that the increase in the dissolved copper LC50 is due primarily to the reduction in the toxicity/unit concentration of one or more toxic copper species with increasing hardness. The reason is that in going from the ambient lake water to the 2×10^{-3} M $CaCl_2$, water, the combined increase in the concentrations at the LC50 point of Cu^{+2} and all other major inorganic copper species is much greater than the calculated copper-organic concentrations in either water (see Table 3-2). Therefore, it is unlikely that any reduction in the concentrations of copper organic complexes could satisfy equation (1-14) alone without some reduction in the toxicities/unit concentration of one or more toxic copper species also occurring.

An analysis of the results of set 2 of the copper toxicity tests in Section 3.2 indicated that the toxicities/unit concentration of at least some copper species also decreased with increasing pH, at least over the range of pH 6.6 to pH 8.0. The increase in the dissolved copper LC50 with increasing pH over that range appears to be at least partially due to the decrease in the proportion of Cu^{+2}. It may also be due to the decrease in the copper organic proportion which may be caused by the increased competition between CO_3^{-2}, OH^-, and organic ligands for Cu^{+2}. However, the increase in the dissolved copper LC50 is also probably at least partially due to a reduction in the toxicity/unit concentration of one or more toxic copper species with increasing pH. The reason is that despite the decrease in the proportion of the Cu^{+2}, the concentration of Cu^{+2} at the LC50 point remains relatively constant, whereas the concentrations of all of the other major inorganic species greatly increase with increasing pH (see Table 3-2). Therefore, it is unlikely that any decrease in the concentration of the much smaller copper-organic fraction over that pH range could satisfy equation (1-14) alone without some reduction in the toxicities/unit concentration of one or more toxic copper species also occurring.

An analysis of the results of set 4 and set 5 in Section 3.4 and 3.5 indicated that the presence of added humics either increased the toxicity/unit concentration of one or more toxic copper species, or formed toxic complexes with copper, or both. The reason is that in going from ambient lake water to any of the waters with added humics, the concentration at the LC50 point of Cu^{+2} and all other major inorganic copper species decrease. Therefore, in order for equation (1-14) to be fulfilled, the toxicity/unit concentration of one or more toxic copper species must increase in the presence of humics and/or the humics must form at least some toxic complexes with copper.

It has been shown that changes in the proportions and concentrations of inorganic copper species can often not account for the differences between LC50 values for different test waters. However, it is conceivable, at least from the standpoint of fulfilling equation (1-14), that such changes could fully account for the differences between the LC50 values for the two highest pH waters of set 2, the three waters of set 3 at higher than ambient alkalinity, and the three highest alkalinity test waters of set 6. There does not appear to be any significant copper-organic complexation in any of those waters, nor is there any substantial evidence to indicate that the toxicities/

unit concentration of copper species vary between the waters. However, our attempts to determine values for the toxicities/unit concentration of the inorganic copper species through multiple linear regression on the data for those test waters was unsuccessful as discussed in Section 3.11. One possible explanation is that the toxicities/unit concentration of at least some of the copper species may have varied between the waters as a function of pH and/or alkalinity. However, the failure could have also been due to a possible change in the sensitivity of the organisms to copper toxicity with time.

The postulate that the sensitivity of the organisms to copper toxicity is changing with time is based on Figure 3-7. Figure 3-7 shows that in going from the ambient lake water in set 1 to that of set 3 and set 4, the dissolved copper LC50s increase monotonically by a factor of over 3, while the Cu^{+2} LC50s increase monotonically by a factor of over 5. Therefore, it appears that the sensitivity of fathead minnow larvae to copper toxicity was decreasing with time, which would be reflected by an apparent decrease in the toxicities/unit concentration of copper species. However, it should be noted that the ambient lake water of set 1, in which the lowest dissolved copper LC50 and Cu^{+2} LC50 were observed, was also calculated to have by far the highest copper-organic fraction among the ambient lake waters. Therefore, it is possible that variations in the copper-organic fraction in ambient lake water could account for some of the variations in the dissolved copper and Cu^{+2} LC50s.

4.2 POTENTIALLY TOXIC COPPER-ORGANIC COMPLEXES

There was substantial evidence presented in Section 3.10 to support, though not conclusively, the postulate that substantial copper-organic complexation can occur in Lake Superior water at or below ambient pH and/or alkalinity despite the relatively low organic content of Lake Superior water of approximately 1 ppm TOC. The evidence included the magnitude of the steeper than Nernstian slopes of the electrode response in ambient lake water and lake water with lowered pH and/or alkalinity (see Table 3-6), the copper titrations of Lake Superior water discussed in Section 3.10, and the computer calculations of copper-organic complexation presented in Table 3-7 which used glycine as a substitute for organic nitrogen in Lake Superior water.

Of course, there is still a possibility that the observed differences between dissolved copper and the sum of inorganic copper concentrations based on electrode determinations of Cu^{+2} activity in lake waters with ambient or lower pHs and/or alkalinities, are due to interferences with the electrode. However, the major known chemical interferences with the electrode do not appear to be at high enough concentrations in Lake Superior water to cause a significant effect. The agreement between electrode determinations of Cu^{+2} concentrations and REDEQL computations of Cu^{+2} concentrations based on inorganic speciation alone for some ambient lake waters and almost all lake waters with elevated pH and/or alkalinity is good with ratios varying from 0.85 to 1.15, as can be seen in Table 3-6. Also, the slopes of the electrode response in such waters, particularly the ones with elevated pH and/or alkalinity, are generally close to Nernstian as can also be seen from Table 3-6.

The possibility that the large differences between the slopes of the electrode response in waters with lower than ambient pH and/or alkalinity and in waters with higher than ambient pH and/or alkalinity was due to either an ionic strength or pH effect was also investigated. However, the addition of 6×10^{-3} M $NaNO_3$ to ambient lake water had virtually no effect on the non-Nernstian response of the electrode in lake water (see Section 3.10), which indicated that large increases in the ionic strength without an associated increase in alkalinity does not modify non-Nernstian responses. Also, even though the slopes of the electrode response are greatly dependent upon pH in Lake Superior water, pH does not appear to directly effect the slope of the electrode response since Nernstian slopes can be obtained at both low pH in 0.01 M acetate buffer or at higher than ambient pH in lake water.

The above discussion indicates that the large differences in the slope of the electrode response in lake water as a function of pH and alkalinity may be due to the dependence of copper-organic complexation on those variables. In particular, the amount of copper-organic complexation may decrease with increasing pH and/or alkalinity due to the increased competition between OH^-, CO_3^{-2}, and organic ligands for Cu^{+2}. Although increasing pH will decrease the competition of H^+ with Cu^{+2} for organic ligands, the copper-organic complexation does appear to decrease with increasing pH, at least at pHs above 6.6.

Therefore, the decrease in the competition between H^+ and Cu^{+2} for organic ligand with increasing pH above 6.6 does not appear to be sufficient to offset the increased competition between OH^-, CO_3^{-2}, and organic ligands for Cu^{+2}.

Although there is substantial evidence to support the postulate that significant amounts of copper-organic complexation can occur in lake waters with ambient or lower alkalinities and/or pHs, it is not known whether such copper-organic complexes contribute significantly to toxicity. The reason is that in each case in which the assumption of the presence of toxic copper-organic complexes can help explain the differences between the toxicities of two test waters, an assumption that the toxicities/unit concentration of the inorganic species change can explain the differences equally well. The reverse is not always true because in some cases it is apparent that neither changes in the proportion of inorganic copper species nor in copper-organic species can completely account for the differences in the LC50 values between two test waters. That can be seen from the earlier discussion on the apparent decrease in the toxicities/unit concentration of copper species with increasing hardness and/or pH.

4.3 CONCLUSIONS WITH RESPECT TO THE FEASIBILITY OF DEVELOPING A CHEMICAL SPECIATION METHOD FOR PREDICTING COPPER TOXICITY IN SITE WATERS

The results of our work, as summarized in Sections 4.1 and 4.2, indicate that the feasibility of developing a chemical speciation method for predicting LC50 values in site water from non-site toxicity data bases and the average site specific values of general water quality parameters is very low. The results have shown that differences in the LC50s between two test waters can often not be fully explained by changes in the proportion or concentration of inorganic copper species alone. Therefore, it appears that changes in the toxicities/unit concentration of at least some toxic copper species and/or changes in the proportions or concentrations of toxic copper-organic complexes also occur between such waters. Unfortunately, the chemical speciation method is not well suited to handle either possibility.

As previously discussed in Section 3.1, the apparent decreases in the toxicities/unit concentration of toxic copper species with increasing hardness could possibly be handled by determining several sets of toxicities/unit concentration, one for each of several values of hardness covering the range of hardness normally encountered in natural waters. However, such procedures would be subject to large error by the restricted ranges of hardnesses used for the determination of each set of toxicities/unit concentration. Although the problem with hardness could possibly be approximately handled, the apparent decreases in the toxicities/unit concentration of copper species with increasing pH, at least in lake waters with ambient or lower alkalinity, cannot be handled by the chemical speciation method unless the toxicities/unit concentration of all of the copper hydroxy species are negligible which is unlikely. The reason, as discussed in Section 3.2, is that to determine the toxicities/unit concentration of copper hydroxy species, it is necessary to vary the proportion of such species with respect to Cu^{+2} which can only be done by changing the pH. However, if in changing the pH, the toxicities conentration of the copper hydroxy species are also changed, the values of the toxicities/unit concentration cannot be determined.

Although apparent changes in the toxicities/unit concentration of copper species may instead, in some cases, be at least partially due to changes in the concentrations of toxic copper-organic complexes, the presence of toxic copper-organic complexes is potentially equally detrimental to the development of a chemical speciation method. The reason is that if more than a few copper-organic complexes contribute significantly to toxicity, it would probably be impossible to identify all such complexes, calculate the associated ligand activities and determine the toxicities/unit concentration of all of the complexes. Even if that could be done for Lake Superior water, the types of copper-organic complexes in another water would probably be much different. The same problem will also be encountered if copper-humic or copper-clay complexes are toxic.

As previously discussed, the differences between LC50 values for some of the test waters could be explained by changes in the proportions or concentrations of inorganic copper species alone without postulating that the differences were also due to changes in toxicities/unit concentration and/or in the proportions or concentrations of toxic copper-organic species.

However, our attempts to determine the toxicities/unit concentration of the major inorganic copper species from a multiple linear regression on the data for those test waters were unsuccessful. It is not known whether the failure to make such determinations was due to any changes in the toxicities/unit concentration with pH, alkalinity, or time. It is also possible that the failure was due to the inability of a simple additive non-synergistic, non-antagonistic model as represented by equation (1-10) to explain toxicity. However, there is insufficient data available to attempt to formulate a more complex model.

4.4 CONCLUSIONS WITH RESPECT TO THE FEASIBILITY OF DEVELOPING A TOXICITY FACTORS METHOD FOR PREDICTING COPPER TOXICITY IN SITE WATERS

The feasibility of developing a toxicity factors method for predicting LC50 values in site waters from non-site toxicity data bases and the average site specific values of general water quality parameters is greater than for developing a chemical speciation method. The reason is primarily because any changes in the toxicities/unit concentration of toxic copper species with the change in a general water quality parameter should affect, and be reflected by, the functional dependence of copper toxicity (e.g., LC50 values) on the general water quality parameter causing the change (e.g., pH). However, despite the greater feasibility of developing a toxicity factor method, there may be major potential problems with its development as well.

The major potential problems with the development of a toxicity factors approach are the possibilities that numerous copper-organic complexes and/or copper-humic complexes and/or copper-clay complexes are toxic. If that is the case, it would be impossible to accurately predict toxicity in a site water from general water quality parameters alone, since the types and characteristics of such complexes would vary greatly from site to site. However, even if such complexes are toxic, they do not appear to be toxic enough to make criteria based on total or dissolved copper LC50 values non-protective. That can be seen from the fact that dissolved copper LC50 values increase with increasing organic and/or suspended solids concentrations. Therefore, if such complexes are shown to exert significant toxicity, total or dissolved copper should probably be the measured form of copper chosen on which to base LC50s in the toxicity factors method. Although those measurable forms of copper are probably much greater than the bioavailable fraction, criteria based on them will probably be protective in the wide variety of site waters which can be encountered.

If it is shown that the copper-humic and copper-clay complexes are non-toxic, the accuracy of the toxicity factors methods in predicting toxicity in site waters could probably be greatly improved by basing LC50s on a measurable form of copper which could approximate the bioavailable fraction better than dissolved or total copper, such as perhaps >1,000 molecular weight exclusion ultrafiltration. For example, if humics and suspended clay affect copper toxicity primarily by decreasing the bioavailable fraction through binding and adsorption, basing LC50s on some measurable form of copper which closely approximates the bioavailable fraction should make such LC50s much less dependent on humic and suspended clay concentrations than total or dissolved copper LC50s. In addition, if the lower molecular weight copper-organic complexes are also found to be relatively non-toxic compared to inorganic species, it might be advantageous to base LC50s on the sum of all major inorganic coppercomplex concentrations rather than 1,000 molecular weight exclusion since such LC50s would be much less dependent of both high and low molecular weight organic content. However, LC50s should probably not be based on Cu^{+2} alone due to the apparent non-monotonic dependence of Cu^{+2} LC50s on pH in water with moderately low alkalinities as is shown in Figure 3-B.

4.5 RECOMMENDATIONS

The results of copper toxicity tests so far have often shown that differences in copper toxicity between different test waters cannot be fully explained by changes in the proportions or concentration of inorganic copper species alone, and that changes in the toxicities/unit concentration of toxic copper species and/or changes in the proportions or concentrations of toxic copper-organic complexes probably also generally occur. Although, as discussed previously, it is not possible from the present data to determine the relative contributions of changes in toxicities/unit concentration and changes in the proportions or concentrations of toxic copper-organic complexes to toxicity changes, either case is extremely detrimental to the development of a chemical speciation method. Furthermore, it appears likely that changes in several of the general water quality parameters do change the toxicities/unit concentration of at least some of the toxic copper species. Also, attempts to determine the toxicities/unit concentrations of copper species, from multiple linear regression uaing data from test waters in which no obvious changes in the toxicities/unit concentration had occurred, were unsuccessful. Therefore, it is strongly recommended that efforts to develop a chemical speciation method be dropped in favor of developing a toxicity factors method. That does not necessarily mean that all work with the curpic ion selective electrode should be dropped. As mentioned previously, some linear combinations of the concentrations of copper species calculated from electrode measured Cu activities could be a useful measurable form of copper on which to base LC50s in the development of a toxicity factors method.

If copper-humic and copper-clay complexes are shown to be relatively non-toxic, efforts could be concentrated on the development of a toxicity factors method which uses, if possible, a measurable form of copper which will minimize or hopefully eliminate the dependence of the LC50 values on humic and suspended clay concentrations. However, realistically, even if such a method is developed, it may not be well received if the measurable form of copper chosen is much more difficult to detenmine than dissolved copper. Therefore, as a possible alternative, EPA should consider using dissolved copper as the measurable form of copper on which a toxicity factors method should be based. Of course, dissolved copper LC50s are very dependent upon humic and suspended clay concentrations. Furthermore, the types and characteristics of humics and clay vary so much, it would be very difficult to try to model such effects. Therefore, if EPA decides to use dissolved copper as the measurable form of copper on which to base the toxicity factors method, it should be realized that any resultant criteria derived would probably be extremely conservative. Nevertheless, if current equations relating total and dissolved copper LC50s to hardness can be extended to multivariable equations relating total or dissolved copper LC50s to other general water quality parameters as well such as pH, any resultant criteria should be less conservative than they are now.

The effects of alkalinity on copper toxicity appear to be small compared to hardness as can be seen from a comparison of the results of set 1 and set 6 of the copper toxicity experiments. Therefore, since hardness and alkalinity are so closely correlated in natural waters, it might be useful to break hardness into carbonate hardness which would include any effect of alkalinity, and non-carbonate hardness instead of considering alkalinity as a separate variable.

The development of a multivariate equation relating LC50s to hardness and pH or possibly carbonate hardness, non-carbonate hardness, and pH should be based on the results of toxicity tests run in water which have typical combinations of carbonate hardness, non-carbonate hardness, and pH values. In general, waters will have a hardness equal to or greater than alkalinity so that most waters will have some non-carbonate hardness. In addition, most waters will have a pH somewhat lower than that expected if they were in equilibrium with the atmosphere. Therefore, the tests should be run in waters at several different hardnesses equal to the alkalinity covering the range of alkalinity normally encountered in natural waters, and at pHs equivalent to equilibrium with the atmosphere. In addition, tests should be run in waters with non-carbonate hardness added in the form of Ca^{+2} and Mg^{+2} chloride and sulfate salts, and pH lowered with CO_2 bubbling. The base waters in which the hardness is equal to the alkalinity and the pH equivalent to that of equilibrium with the atmosphere should be made with mixtures of calcium and magnesium bicarbonates at a Ca^{+2}/Mg^{+2} ratio typical of natural waters and then aerated. Furthermore, since recent work by Benoit and Mattson (83) have indicated that chloride and sulfate salts may exert somewhat different effects on toxicities, the non-carbonate hardness should be added in ratios of Cl^{-}/SO_4^{-2} salts similar to the Cl^{-}/SO_4^{-2} ratios typically seen in natural waters. Because the effects of Na^{+} and K^{+} are not well known, the use of Na^{+} and K^{+} salts should be avoided. The use of such salts is actually not necessary if hardness is divided into carbonate and non-carbonate hardness and the effects of alkalinity is considered jointly with hardness in the form of the carbonate hardness. Typical combinations of carbonate hardness, non-carbonate hardness, and the pH can be determined from national surveys of freshwaters such as the U.S. Geological Survey entitled Quality of Rivers of the United States, 1975 Water Year. Typical ratios of Ca^{+2}/Mg^{+2} and Cl^{-}/SO_4^{-2} can also be determined from such sources.

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